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Contents

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On the Retinal Representation in the Lateral Geniculate Body.....*Tadashi Fujino & Yonan Go*

Fluctuation of the Fat in Human Cornea with Age and Experimental Studies on the Process of the Appearance of Fat.....*Yoshio Kaneko*

A Study on the Genesis of the Spiral Nerve Ending in the Extrinsic Ocular Muscle.....*Tamae Okamoto*

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Studies on Night Myopia.....*Hiroshi Murata*

Studies on Herpes Cornea. 1. Biomicroscopic and Histologic Findings on the Experimental Herpetic Keratitis.....*Shusaku Kitano, Jung-Shien Liu, and Noriomi Ochi*

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Electromyographic Study on Retraction Syndrome.....*Soichiro Sato*

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12

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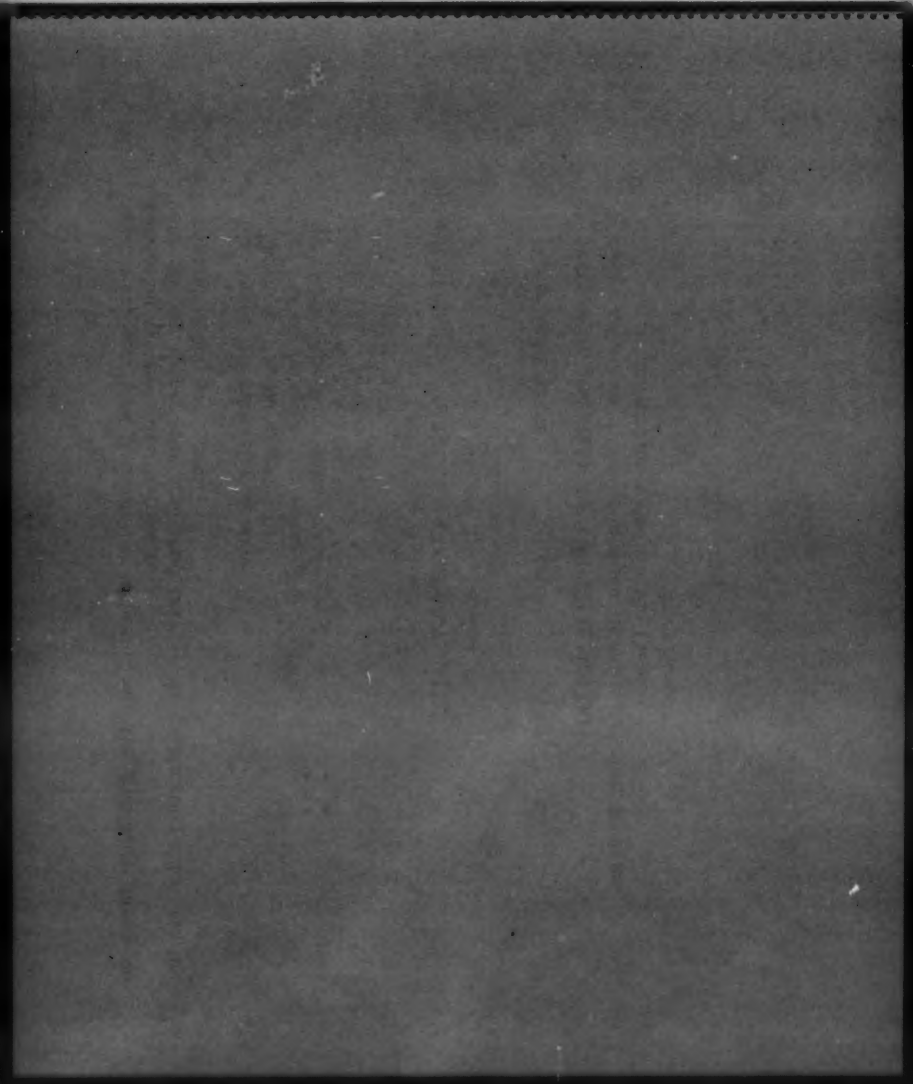

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Contents

On the Retinal Representation in the Lateral Geniculate Body····· *Tadashi Fujino & Yonan Go*··· 1

Fluctuation of the Fat in Human Cornea with Age and Experimental Studies on the Process of the Appearance of Fat····· *Yoshio Kaneko*··· 8

A Study on the Genesis of the Spiral Nerve Ending in the Extrinsic Ocular Muscle····· *Tamae Okamoto*···12

Studies on Allergic Reactions caused by Lens Substances. 1. Studies on the Organ Specificity and the Antigenic Structures of Lens Proteins····· *Takashi Mizukawa, Shiro Uyama, Reiko Yoshida, Yasuo Mimura, and Shinkichi Kizu*···16

Studies on Night Myopia····· *Hiroshi Murata*···22

Studies on Herpes Cornea. 1. Biomicroscopic and Histologic Findings on the Experimental Herpetic Keratitis····· *Shusaka Kitano, Jung-Shien Liu, and Noriomi Ochi*···31

Pathohistological Studies on Arachnoiditis, with especial Reference to Circuration Disturbance of the Cerebrospinal Fluid····· *Jo Imachi and Ippei Kajikawa*···37

Electron Microscopic Observation of the Optic Nerve····· *Yoshiaki Yuri*···48

Electromyographic Study on Retraction Syndrome····· *Soichiro Sato*···57

Studies on the Development of the Depth Perception for Near and Diatant Object in the Childhood····· *Jisuke Kitao*···67

ON THE RETINAL REPRESENTATION IN THE LATERAL GENICULATE BODY

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Introduction

Retinal representation of the lateral geniculate body was first demonstrated by Brouwer and Zeeman in 1926, and has received world wide recognition. There are, however, some questions unanswered. For instance, in regard to presentation of the crossed and uncrossed fibers in the lateral geniculate body Minkowski (1920) insists that individual layer is projected seperately, while Brouwer and Zeeman reject its possibility. Literatures concerning this problem decline to stand on either side simply admitting the existence of two contradictory theories.

Years of our experimental study on this problem were rewarded by clarification of this tangled question, on which we are happy to report here.

Experimental Method

Sixteen Macaca cyclopses were subjected for the experiment. Unilateral removal of the eye was performed or partial retinal lesions were produced by diathermal coagulation of the eye under ether anesthesia, and after a certain interval these animals were sacrificed by administration of fatal dose of ether or Nembutal sodium. The cerebrum with opticus and bulbus was removed from the dead animal carefully avoiding unnecessary injuries. The specimen was emerged and fixed in 10% formalin, Mueller's solution, or absolute alcohol depending on the purpose of individual study. In order to study Marchi's granules the specimen was fixed at times in Orth's solution, paying special attention to prevent growth of false granules.

Serial sections of 25 to 30 micron were made from the celloidin block and stained by Nissl's method (thionine, metylenblue or toluidineblue), myelin-sheath staining method (Weigert-Pal, Sugamo, Sugamo-carmine, and Weil), or Marchi's method. In some specimens frozen sections were made from the block without delay and stained by silver impregnation method (Glees and Clark). Wax or paper reconstruction models were made with the aid of Abbé's drawing instrument when needed.

Results

Experiment 1. Morphology of the lateral geniculate body of normal monkeys

Three monkeys were employed for this study: Monkey 1, an adult monkey (3.5 kg.) with no abnormality in retina; Monkey 2, a young normal monkey (1 kg.) and another monkey, Monkey 3 (7 kg.), who died during the experimental surgery of retina.

Findings. The lateral geniculate body presents in frontal section the shape of a

hoof with its convex side to the dorso-lateral direction, showing six layers of cell strata in Nissl's stain. The ventral or lowest two layers are composed of large cells and the remaining four dorsal layers are of small cells.

Experiment 2. The lateral geniculate body of the monkey following removal of an eye, Nissl's stain

Subjects of this study were the following animals: Monkey 4 (4.5 kg.), adult monkey whose left eye was removed 14 months prior to autopsy; Monkey 5 (3.5 kg.), an adult monkey whose left eye developed phthisis and who was sacrificed 23 months after the operation; and Monkey 6 (0.83 kg.), a young monkey whose right eye was removed 39 months prior to autopsy.

Findings. Nissl's stain of the lateral geniculate body of the normal side reveals normal second, third and fifth layers and abnormal first, fourth and sixth layers of abnormally small cells with poor chromatin and marked atrophy of the cells. On the other hand, the lateral geniculate body of the operated side has atrophy in the second, third and fifth layers. Figure 1 illustrates a frontal section of the left lateral geniculate body in thionine stain, demonstrating the distinct difference between the normal and the atrophic cells. Reconstruction model of this normal cell layer is shown in Figure 2, in which the right is the oral side and the left is the caudal side.

Experiment 3. Change in Marchi's stain specimen of the lateral geniculate body whose host had unilateral removal of the eye.

Animals subjected to this study were: Monkey 7 (3.5 kg.) and Monkey 8 (3.6 kg.) whose left eyes were removed 21 days prior to autopsy and the serial frontal sections of the lateral geniculate bodies were stained by Marchi's method.

Findings. Marchi's granules are diffusely scattered over the lateral geniculate bodies bilaterally without disclosing distinct cell layers as seen in Nissl's stain, although there is some difference of density in the distribution of granules, suggesting the presence of strata. Marchi's granules are distinctly visible in the areas corresponding to the three normal cell layers found in the Nissl's stain specimen of the second experiment. In other words, distinction between the crossed and uncrossed fibers is not clear.

Experiment 4. Change in the lateral geniculate body following injuries on various areas of the retina

The normal retina of adult monkeys were cauterized in different areas to produce injury lesions 21 days prior to autopsy, and serial frontal sections were stained by Marchi's method.

Retinal damage was produced on the macular region of Monkey 9 (4.9 kg.) and Monkey 10 (5.0 kg.), and on the nasal periphery of Monkey 11 (3.5 kg.) and Monkey 12 (4.0 kg.), and on the lower periphery of Monkey 13 (1.8 kg.) and Monkey 16 (4.5 kg.), or eight monkeys in total.

Findings. Marchi's granules are recognized in the course of optic fibers which belong to the damaged area of the retina.

1. Damage to the nasal periphery—Presence of Marchi's granules is limited to the medial and lateral sides of the ventral portion of the oral half of the right lateral geniculate body. It is not found in the caudal half, dorsal portion, or in the left lateral geniculate body.

2. Damage to the temporal periphery—The same findings as in the case of nasal periphery damage except that Marchi's granules disappear in the right instead of the left lateral geniculate body.

3. Damage to the lower periphery—The lateral geniculate body demonstrates Marchi's granules in the oral and ventro-lateral side bilaterally. No granule is present in the medial side or in the dorso-caudal portion.

4. Damage to the macular region—Marchi's granules are distributed bilaterally in subtotal area of the oral portion of the lateral geniculate body. It is also found in a large area of the caudal portion and the number is significantly greater in comparison with other lesions of damage. It is regretful that poor staining technic interfered with further study of details.

Experiment 5. Small bundle formation of the optic fibers in the lateral geniculate body.

During the previously described experiment we noticed a rather striking finding of optic fibers forming small bundles of several hundreds of fibers. There is no reference on this subject in the literature as far as we could follow. Therefore, we have proceeded to study them. Various staining methods were employed on the samples selected at random from the above specimens; hematoxylin-eosin, azan, Mallory's, Nissl's, myelin-sheath, glia stains and silver impregnation method.

Findings. Small bundles are indistinguishable from each other in myelin-sheath stain which stains whole area diffusely dark, they are distinguishable in hematoxylin-eosin and Nissl's stain only by recognizing the rings of glia cells. The number of small bundles was 3,600 in one frontal section of the left lateral geniculate body of adult monkey (Monkey 1), a region close to the oral pole. Calculation was made possible by magnified microphotographs taken seperately in small areas and arranged to show the entire field at one glance, and then the bundles were counted meticulously. Since tract fibers are believed to reach 600,000 to 1,000,000, the fibers contained in one small bundles must be 160 to 270. This figure does not differ in the oral part from in the oral pole, but it decreases in the caudal part until finally they disappear in the vicinity of the caudal pole. This indicates that the group of fibers forming small bundles gradually spread and end at the lateral gediculate body. In silver preparation of sagittal sections many small bundles are found running horizontally, as shown in Figure 3, in which also is seen the bloomshape spreading of the small bundle terminals. Nissl's stain does not stain the interior of the glia cell ring, but myelin-sheath stain reveals distinctly fiber bundles in this area, as shown in Figure 4.

Discussion

Retinal representation of the crossed and uncrossed fibers in the lateral geniculate body.

Modern theory on this subject was established by Minkowski in 1920. He discovered the six layers of the lateral geniculate body in normal Macaca rehsus and also found degeneration of cells in specific layers stained by Nissl's method 8 months after removal of an eye. For example, atrophy of cells took place in the first, fourth and sixth layers of the right lateral geniculate body after the right eye had been removed. He confirmed this fact in the brains of three unilaterally blind men, according to his report.

Only six years later, however, Brouwer and Zeeman (1926) concluded that the crossed and uncrossed fibers do not represent in specific layers of the lateral geniculate body based on their finding of Marchi's granules over the entire areas of bilateral lateral geniculate bodies of a monkey whose one eye had been removed previously.

Next opponent of Minkowski was Henschen (1926) who described many clearly outlined normal cells in the layer of atrophic cells and degenerated cells in the layer of normal cells in Nissl's stain specimen of four unilaterally blind persons, thus contradicting to Minkowski's theory which assserts distinctly different layers of all normal cells and of all atrophic cells. Henschen's paper rang a toll for Minkowski's theory, and Brouwer and Zeeman's theory gained its popularity.

A decade later a hord of supporters of Minkowski came to his resque. They were v. Santha (1934), Penman (1934), Barris (1935), Horanszky (1936) and Glees and Clarke (1941). They gave support to Minkowski's theory on the basis of their findings in human or monkey lateral geniculate body treated by Nissl's method, coinciding with Minkowski's finding instead of Henschen's.

However, one question remained unsolved, i.e., why degenerative granules in Marchi's preparation scatter diffusely without forming layers in the lateral geniculate body of the monkey whose one eye had been removed. This is the very question with which Brouwer and Zeeman challenged Minkowski. Penman, one of the supporters of Minkowski, believes the inadequacy of Marchi's method for this type of study, but it was not a convinsive argument. None of other workers refers on this doubt or try any other method than Nissl's method which we believe should be reevaluated.

As mentioned above, we found in our second experiment distinctive atrophy in three layers of the lateral geniculate body stained by Nissl's method (Fig. 1 and 2). This contradicts with Henschen's finding and fully agrees with Minkowski and his supporter's results. In Marchi's stain, as proved in our third experiment, degenerated granules appear diffusely in the entire area of the lateral geniculate body without forming layers, although their number decreases in the vicinity of caudal pole as compared with the oral pole. This finding agrees with Brouwer and Zeeman's result.

We had been puzzled by these two contradictory findings which annoyed our predecessors likewise, until we studied silver preparation of sagittal sections (Experiment 2,

Fig. 1. Monkey 4. Middle part of left geniculate body, frontal section, thionine-stain. ×45.

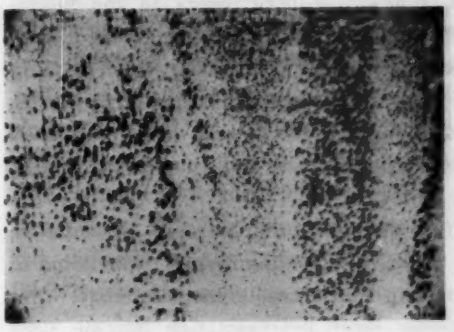


Fig. 2. Ditto. Reconstruction model.

Fig. 3. Monkey 5. Left geniculate body, sagittal section, silver method. The right is the oral side.

Fig. 4. Monkey 1. Middle part of left geniculate body, frontal section, Heidenhein's myelin-sheath stain. ×41.

Fig. 5. Monkey 5. Right geniculate body, sagittal section, thionine stain. ×11.

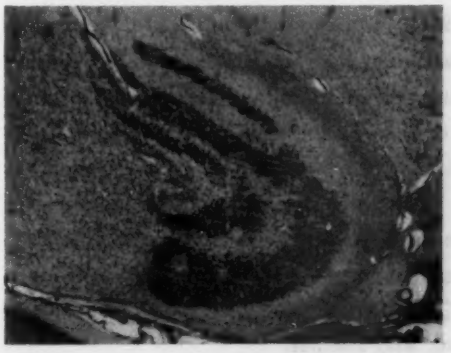
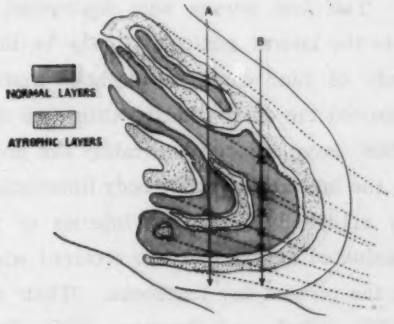


Fig. 6. Ditto. Schema.



Monkey 5), which revealed numerous optic fibers divided longitudinally in nearly all areas of the lateral geniculate body with each fiber penetrating through individual layer, coinciding with Nissl's stain specimen. This relation of optic fibers to the lateral geniculate body means that after single eye removal many Marchi's granules appear in the area corresponding to the normal cell layer (marked "X" on the line "B" in Fig. 6) of the specimen sectioned at "A" and "B" in Figure 6. In other words, Marchi's granules are diffusely distributed in any part of the lateral geniculate body decreasing gradually when they approach from the oral part to the caudal part. Our finding supports this logical course of the optic fibers.

In short, we uncovered the mystery of diffuse distribution of Marchi's granules in the lateral geniculate body which was the last opposition to Minkowski's explanation of representation of the crossed and uncrossed fibers in the lateral geniculate body. We believe, therefore, Minkowski's theory of specific layer representation became fully acceptable.

Small bundle formation of the optic fibers in the lateral geniculate body.

As already described in the chapter on Experiment 5, we were unable to find any reference in literature on this small bundle formation of the optic fibers. Taboada (1928) is the only one who studied Golgi specimen of the lateral geniculate body of a chimpanzee and described bundle formation of the optic fibers directed towards the cells spreading their ends in brush or basket form, so-called "terminal arborization", embracing a group of cells which count 12 to 15 in the ventral side and 60 to 100 in the dorsal side. These figures differ from our figure of 160 to 170, although he agreed with us by recognizing bundle formation of the fibers as units. This difference is being evaluated in human lateral geniculate body which is still in progress. As described before, our small bundles run from the optic tract to the lateral geniculate body and then to the caudal direction showing bloom shape spreading in many places, probably forming terminals on its course of spread.

Projection of various parts of the retina in the lateral geniculate body.

The first person who discovered the projection of particular section of the retina into the lateral geniculate body is Rönne (1914), who studied the lateral geniculate body of four cases of alcoholic amblyopia and one case of diabetic amblyopia and reported the dorso-caudal localization of macular representation. Brouwer and Zeeman (1926) have mapped accurately the projection of the macula and its surrounding area in the lateral geniculate body illustrating its schema. This work was done on 16 monkeys by artificially producing injuries of various parts of the retina. This marvelously conducted experiment has received wide appreciation and their illustration is still used in the present-day textbooks. Their study was approved by many followers: Juba (1933), Clark and Penman (1933), Putnam (1926), Biemond (1929) and Polyak (1933). Our results agreed grossly with that of Brouwer and Zeeman.

Summary

Retinal representation in the lateral geniculate body was studied experimentally on 16 Macaca cyclopses leading to the following results.

1. Retinal representation of the crossed and uncrossed fibers in the lateral geniculate body has two theories: Minkowski's specific layer presentation theory and Brouwer and Zeeman's opposing theory. Findings in Nissl's stain specimen proved the superiority of the former. Diffuse presence of Marchi's granules in the lateral geniculate body after single-eye removal was the reason of opposition to Minkowski's theory, but the meaning of this fact was unknown to any one. We have found that the optic fibers run diffusely in the lateral geniculate body from the oral side to the caudal side. This is why the granules were present in the normal cell layers of the lateral geniculate body in Marchi's stain following removal of one eye. This finding made it possible to accept Minkowski's theory without hesitation.

2. The optic fibers form small bundles in the lateral geniculate body. The are predominant in the oral part of the lateral geniculate body, counting 3,600 in one sample, and decrease in the caudal side disappearing completely at the caudal pole. This finding is ascribed to gradual termination of small bundles in the lateral geniculate body.

3. Representation of various areas of the retina in the lateral geniculate body was studied by Marchi's method, and the findings obtained agreed grossly with that of Brouwer and Zeeman.

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FLUCTUATION OF THE FAT IN HUMAN CORNEA WITH AGE AND EXPERIMENTAL STUDIES ON THE PROCESS OF THE APPEARANCE OF FAT

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As for the fat in the cornea, the arcus senilis and fatty degeneration in human eye and the process of its appearance in the rabbit fed with fatty diet have been studied by various investigators following after Canton (1850)[2], Kamocki (1893)[3] Takayasu (1901)[7], and Chuin (1916)[8]. But it is hard to say that these have already been fully studied. On the contrary many problems remain to be solved.

Therefore, the fluctuation of the fat in Japanese cornea is studied histopathologically and also the experiment in the rabbit is practised.

Materials and Methods

The human eye. The extracted eyes from 64 humans in which any corneal disease was not observed macroscopically were used for this study. The ages ranged from 1 to 86.

Round pieces of the cornea including the boundary part between the cornea and sclera were obtained at the twelve o'clock position by the trepan of 10 mm in diameter. They were fixed in 10% formalin, embeded in geratine and cut by the freezing method. The sections were stained with various dyes for fat-staining, such as Daddi's Sudan III, Sudan black B, Nile blue sulfate and oil red 0 respectively. Some sections were stained with hematoxylin and eosin for control. The preparations were divided into classes by age and examined under the light microscope.

The rabbit. Male rabbits of nearly 2 kgr in body weight were sacrificed for this experiment.

Crystalized cholesterol dissolved in olive oil was used for fatty substance in food. Besides experimental parenchymatous keratitis was caused by injection of albumen. Twenty rabbits were divided into four groups. The first group was fed with cholesterol food and the second was produced experimental parenchmatous keratitis in the course of cholesterol feeding. The third group was fed without cholesterol and only produced experimental keratitis, and the fourth was normal control.

The first and second groups were given everyday 200 mg of crystalized cholesterol in 5 ccm of olive oil which was mixed in scums of bean-curds (tōfú-kasu). Thus, hypercholesterosis was caused.

In quantitative analysis, cholesterol in the blood increased rapidly up to neary the 10th day. After 10 days it became about three or four times (150 to 200 mg/dl) as much as that of the normal rabbit (50 mg/dl). Afterwards in some cases it increased rapidly in the same way and in other cases increased slowly. So individual differences

were noted. When diarrhea or jaundice was accompanied and accordingly weakness appeared on the way, cholesterol food was reduced or stoped to be protected from death.

In the second and third groups, 2 or 3 ccm of 3% albumen was injected once a week subcutaneously in a abdominal portion of the rabbit for 4 weeks. After sensitization was performed sufficiently, 0.1 ccm of 3% albumen was injected in the cornea to cause parenchymatous keratitis. When 0.1 ccm of 3% albumen was injected again in the cases where pannus was observed after inflammatory symptoms by parenchymatous keratits disappeared, severe inflammatory symptoms occured in some cases.

Performing the experiment mentioned above, the progress was observed for 70 to 160 days. After that the eyes were extracted, and the cornea was stained by the same method as human cornea and examined under the light microscope.

Observations

The human eye

a) No specific relation with age was observed in the epithelial or endothelial layer. Only in a few cases fine fatty granules were found here and there.

b) In the membrane of Bowman fat was not observed before the twenties. It began to be stained from the thirties and increased from the forties to seventies. The central region of the membrane was remained to be unstainable and the peripheral region was stained intensely in nearly the half. On the other hand, stainability decreased in the eighties.

c) In the stroma of the cornea stained portions arised from the outer and inner layers. Because the peripheral region of the stroma ended obliquely and the fat deposition in the inner layer was found only at the portion under the corneal ring, the stained portion of the peripheral region of the outer layer was remarkable in the twenties to forties (fig. 4). In the forties to fifties both the outer and inner layers were stained in the same portion respectively. In the sixties fatty deposition was rather notable in the inner layer and approached to the central region (fig. 5), but there was no cases where the latter was stained.

d) In the membrane of Descemet, fat appeared in the tens and was found in almost all cases of the twenties. Above the forties all layers of the membrane were stainable in all cases, but the peripheral region was stained in gradations. In the eighties stainability was rather reduced.

The rabbit

a) In the epithelial layer, fat was observed only at the portion which adjoined to the fatty deposition of the stroma in rabbits of the first group fed for 160 days.

b) In the peripheral region of the stroma of the first and second groups, fatty deposition was found regionally at the portion which was continued from nets of the blood vessel around the cornea just under the epithelial layer. The inner layer was

Fig. 1. The cornea of the rabbit in the first group fed for 80 days. Low magnification.

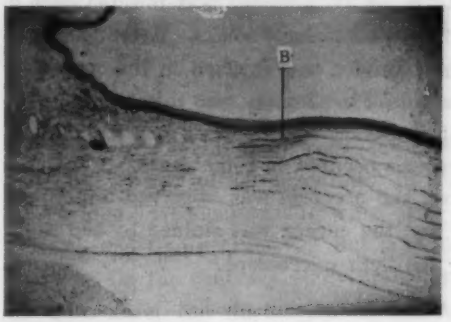

Fig. 2. The cornea of the rabbit in the second group fed for 80 days. Low magnification.

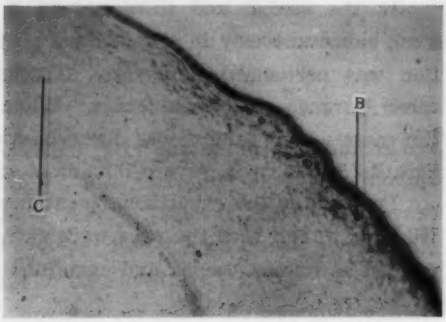

Fig. 3. The cornea of the rabbit in the third group fed for 80 days. Low magnification.

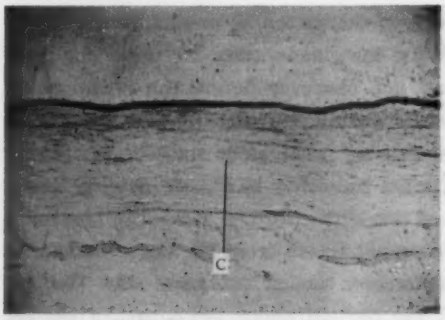

Fig. 4. The human cornea. 30 years old. Female. Low magnification.

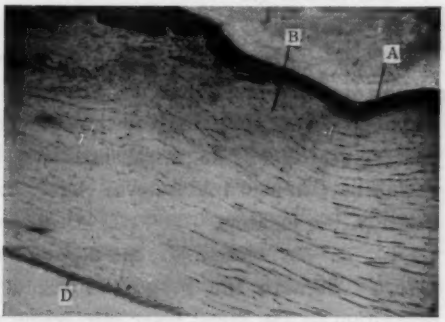

Fig. 5. The human cornea. 70 years old. Male. Low magnification.

A: Stained fat in the membrane of Bowman.
B: Stained fat in the peripheral region of the stroma.
C: Stained fat in the central region of the stroma.
D: Stained fat in the membrane of Descemet.

stained much more weakly than the outer layer. In the third and fourth groups no fatty deposition was observed. In the degree and extent of fatty deposition, there was no difference between the fiirst and second groups (fig. 1 and 2).

c) In the central region of the stroma, a few fine fatty granules were

observed among thin lamellae in the second and third groups, but not observed in the first and fourth groups. The degree and extent of fatty deposition is little larger in the second group than in the third group, although distinct differnce between the both groups was not found (fig. 2 and 3).

d) In the membrane of Descemet and the endothelial layer, no fatty deposition was observed generally in all groups. Fat was found, however, in a few cases where cell infiltration was occured under the endothelial layer by the severe inflammation.

Discussion

The human eye. There have been many studies concerning the fat in the cornea and especially arcus senilis. The fluctuation of the fat in the cornea with age was studied fairly in detail by Rohrschneider[6]. In Japan, however, only Kojima[4] described that the appearance of fat was observed in early time by Sudan III method after modification of Kawamura-Yazaki. The present results on the membrane of Bowman, stroma and membrane of Descemet are similar as compared with Rohrschneider's. But the appearance of fat is observed in earier time.

The rabbit. Experimental studies on the rabbit have been published with different results depending upon experimental methods. Chüin[3] found for the first time a similar condition to arcus senilis in cholesterol feeding and postulated that it was caused by hypercholesterosis. Ogino[5] recognized fatty deposition at the central region of the stroma. In the present experiment, fatty deposition is observed only at the peripheral region of the stroma and not at the central region. It may be for shorter term of cholesterol feeding.

The previous authors who performed the artificial treatment to the cornea of the rabbit fed with cholesterol food, reported the fat deposition in the cornea. In the present experiment it is also found at the central region of the cornea. In the cases of parenchymatous keratitis in the rabbit fed without cholesterol, fat deposition is observed at the central region of the cornea as described by Ogino. At the peripheral region, however, fat is not found.

Summary

1. *The human eye.* The cornea taken from 64 humans 1 to 86 years of age was studied in preparations cut by the freezing method and stained with various dyes for fat-staining, especially Daddi's Sudan III, and obtained following results.

No specific relation with age is observed in the epithelial and endothelial layers. In the membrane of Bowman, stained fat is found at its peripheral region in almost all the cases in the thirties. In the forties to seventies it appears in maximum, but decreases in the eighties. In the stroma, the more widely the stained portion of the inner layer expands, the more highly the degree of stain ability of fat becomes. In other words, in the twenties to forties the stained portion is remarkable in the outer layer. On the contrary stainability is higher in the inner layer after the sixties. In

the membrane of Descemet fat is observed almost all the cases of the twenties. Above the forties all the layers become to be stained, but in the eighties stainability decreases.

Finally fat appears in the twenties and spreads in almost all the layers of the cornea in the forties. The deposition of fat becomes maximum in the sixties, on the contrary it decreases in the eighties.

2. *The rabbit.* Twenty rabbits were divided into 4 groupes. The first group was fed with cholesterol food and the second was produced experimental parenchymatous keratitis by albumen in the course of cholesterol feeding. The third group was fed without cholesterol and only produced experimental parenchymatous keratitis, and the fourth was normal control. The cornea was examined by the same method as in human eye. The results were as follows.

In the peripheral region of the stroma, fat deposition is found only in the first and second groups. In its degree and extent, there is no distinct difference between both groups. In the central region of the stroma, the deposition is observed in the second and third groups. Its degree and extent is little larger in the second group than in the third group, but distinct difference is not found between both groups.

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A STUDY ON THE GENESIS OF THE SPIRAL NERVE ENDING IN THE EXTRINSIC OCULAR MUSCLE

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It has been stated that there is no muscle spindle in the human extrinsic ocular muscle. In 1946 Daniel discovered a spiral nerve ending in the human extrinsic muscle. A hypothesis was made by him that this was equal to the complicated muscle spindle of the skeletal muscle.

Methods and Results

The spiral nerve ending of the human extrinsic ocular muscle on 60 cadavers by use of staining methods silver, and the morphology of the nerve, its size and pathway, whether a small number of nerves or a large amount cling and the manner of clinging, branching and the number of fibers in the nerve, the terminal plate, the nature of the

fibers in the nerve, the relation between the nerve and the endplate and the appearance of the vaginal tunic were studied and presented by me. Since then in a newborn who died five days after birth, a simple spiral nerve ending was found. This introduced an interst in the development of the spiral nerve ending in the fetus. As a result, morphologically, whether the spiral nerve ending had the same spindle as general skeletal muscles was observed. In other words in the 45 fetuses from 2 to 9 months, the nerve fibers of the extrinsic ocular muscle were seen in the third fetal month, the motor endplate at 8 months, but the spiral nerve ending was not seen until the 9th month. In the stillborn a beginning of the spiral nerve ending was seen. From the data seen above it can be stated that in humans the spiral nerve ending of the extrinsic ocular muscle appears just before birth.

Comment

The development of the muscle spindles of the body in general have been studied by Tello (1922), Langworthy (1923, 1924), Frazier (1895), and Hewer (1955) but as far as the muscle spindle of the human extrinsic ocular muscle in concerned it is only in recent years that the spiral nerve ending has been discovered. I don't know any paper concerning that of the fetus.

When comparing the general muscle fibers, nerves and nerve endings of the fetus presented by Hewer to this study there were many points which were found to be extremely different. For example Hewer states that in the 20th week the fetus showed complicated sensory nerve endings in arm and leg muscles and at birth were quite well developd. These sensory nerve endings appear early and are well developed at birth where as the motor nerve endplates develop later. In other words the motor nerve endings start developing in the 26th to the 28th week and do not finish developing even at birth. Therefore the development of the motor nerve endplate and muscle spindle of the arm and leg muscles is exactly the opposite to that of the human extrinsic ocular muscle. In other words the spiral nerve endings which is the sensory nerve of the extrinsic ocular muscle begins to develop directly before birth.

By Hewer the human tongue muscle which is morphologically the same as the human extrinsic ocular muscle is different from the muscles in the extremities. Motor nerve endplate of human tongue muscle is well developed at birth but the complicated sonsory nerve endings are not seen in the 26-28 week after conception. They begin developing near termin. This is similar to human extrinsic ocular muscle by my observation. It is believed that the development of the human spiral nerve ending and motor nerve ending is much later than the general muscles.

Hewer states that the development of the nerve ending of the hands & arms is earlier than that of the leg and the motor nerve endplate of the tongue develops esarly. According to all these facts I assume as follow: the reason why the nerve ending of muscle of some organs develops earlier and the others later is in the order of the necessity of the organs after birth.

Summary

It is discovered by me that the spiral nerve ending, which is believed to be the sensory nerve ending of human extrinsic ocular muscle, begins to develop just before the birth.

The fact that the development of the motor nerve ending of the human extrinsic ocular muscle is earlier than the spiral nerve ending shows that it is possible to think the monocular and binocular movement or the function of binocular single vision develops gradually for the first time after birth. In other words the spiral nerve ending of the human extrinsic ocular muscle is assumed, more surely, to have the same function as the other skeletal muscle spindles.

Fig. 1. Spiral nerve ending seen as a nonmedullated nerve fiber in the human extrinsic ocular muscle.

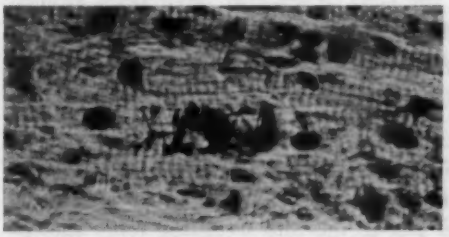

Fig. 2. Thick medullated nerve ébers of the spiral nerve endings seen in the extrinsic ocular muscle. It is clearly seen in this figure that the thick medullated nerve fibers pick the thick muscle fibers as an axis for the spiral. ×700.

Fig. 3. Thin medullated nerve endings in the extrinsic ocular muscle. The nerve fibers are wrapped around the muscle fibers going from one muscle fiber to another. The internal muscle spiral nerve fibers are smaller than of the external muscles.

Fig. 4. Nerve fibers of a three month old fetus.

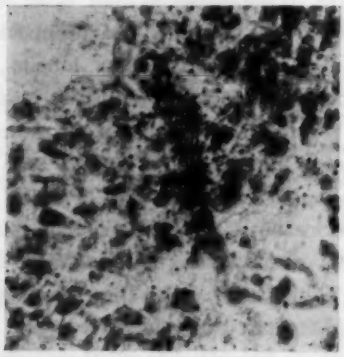

Fig. 5. The transverse lines are clearly seen in the extrinsic ocular muscles of a four month old fetus. The medullated nerve fibers are seen running between and above the muscle fibers along the axis.

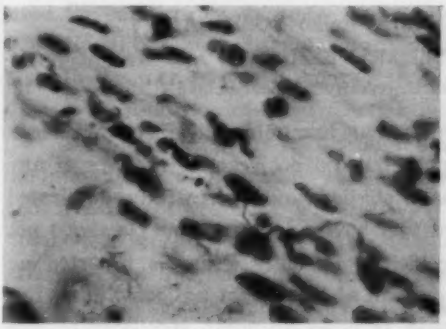

Fig. 6. Extrinsic ocular muscle of eight old fetus. Motor nerve endplate made of medullated nerve fibers are seen.

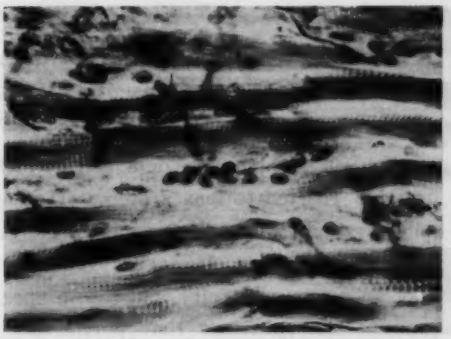

Fig. 7. Simple spiral nerve ending of the extrinsic ocular muscle of a ten month fetus.

Fig. 8. Spiral nerve ending of extrinsic ocular muscle of a newborn.

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STUDIES ON ALLERGIC REACTIONS CAUSED BY LENS SUBSTANCES
1. STUDIES ON THE ORGAN SPECIFICITY AND THE ANTIGENIC STRUCTURES OF LENS PROTEINS

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In 1903, Uhlenhuth demonstrated that an animal immunized against bovine lens proteins reacted to lens proteins of other animals, but did not react to proteins of any other organs. Since then many investigators have studied and successfully duplicated his demonstration. However, Tsutsui reported that bovine lens proteins possessed not only an organ specificity, but also a species specificity. Recently François reported on immunoelectrophoresis that bovine lens proteins had at least eight antigenicities. But no explanation has been made of a relationship between an organ specificity of lens protein and its physico-chemical properties. It is the purpose of this paper to discuss comparatively physico-chemical properties of various animal's lens proteins studied on zone-electrophoresis, N-terminal amino acid analysis and Ouchterlony's agar-gel diffusion technique. Discussion was also made of an antigenic structure of bovine lens cortex and nucleus on immunoelectrophoresis.

Materials and Methods

1. Zone-electrophoresis. Bovine, horse, rabbit, frog lens and human cataractous and normal lens were separated as quickly as possible after death of animals, homogenized in a mortar and extracted with 0.1 M. Na_2HPO_4 and centrifuged at 60000×g for an hour. The supernatant solution was used on a electrophoresis.

2. Separation of the antigens. (bovine lens α-crystallin and β-fraction)

Alpha-crystallin. Water extracts of bovine lens protein (protein concentration: 2 per cent) were adjusted to pH 5.0 with 0.1 N. acetic acid and to this solution ten folds water was added. The precipitates were dissolved in 0.01 N. NH_4OH, then adjusted to pH 5.0 and ten folds water was added. This procedure was repeated three times. The precipitates were lyophilized. This fraction is a pure α-crystallin which showed a single pattern on zone-electrophoresis, moving boundary electrophoresis and ultracentrifugative analysis.

Beta fraction. Water extracts of bovine lens protein were salted out with 50 per cent of ammonium sulfate at pH 7.0 and to a supernatant solution, an equal volume of saturated ammmonium sulfate solution was added. The precipitates were dissolved in a distilled water and dialysed against distilled water. The solution was lyophilized. This fraction was named β-fraction, because it showed a single component in veronal-HCl buffer, but was divided into two components in a phosphate buffer on zone-electrophoresis.

3. Determination of N-terminal amino acid of alpha-crystallin.

Alpha crystallin was dinitrophenylated by Sanger's method. DNP-α-crystallin were hydrolyzed with 6 N.HCl in a sealed tube at 110°C. for 16 hours. The hydrolyzate was adjust to pH 1.0 and extracted with ether and ethyl acetate, then the extracts was used for paperchromatography. As a solvent, 1.5 M. phosphate buffer (1.0M. NaH_2PO_4+ 0.5M.Na_2HPO_4) and butanol saturated with 2 N. NH_4OH. were employed. Developing time in the former was 10 hours and in the latter, 18 hours.

4. Identification of cross reaction between lens proteins of various animals.

As an antigen, 1 per cent protein solution of various animals, and as an antibody, an immunized rabbit serum was employed. Immunized animals were obtained with the following procedures: whole bovine lens extracts, α-crystallin and β-fraction of bovine lens were injected intravenously into rabbits 2 times each week, and seven days after the last injection, the animals were killed and blood was obtained through arteria carotis. The serum was inactivated at 56°C. for 30 minutes and merzonin was added to it. For Ouchterlony's gel diffusion technique, 1 per cent solution of Bactoagar (Difco) in 1 per cent saline was used. The diameter of a well was 2.0 cm and the distance between the edge of central well and that of peripheral well was 1.5 cm. Serum was placed into a central well and antigens of various lens proteins in peripheral wells. Identification was performed in 3 days.

5. Immunoelectrophoretic study.

Immunoelectrophoresis was performed following François' method.

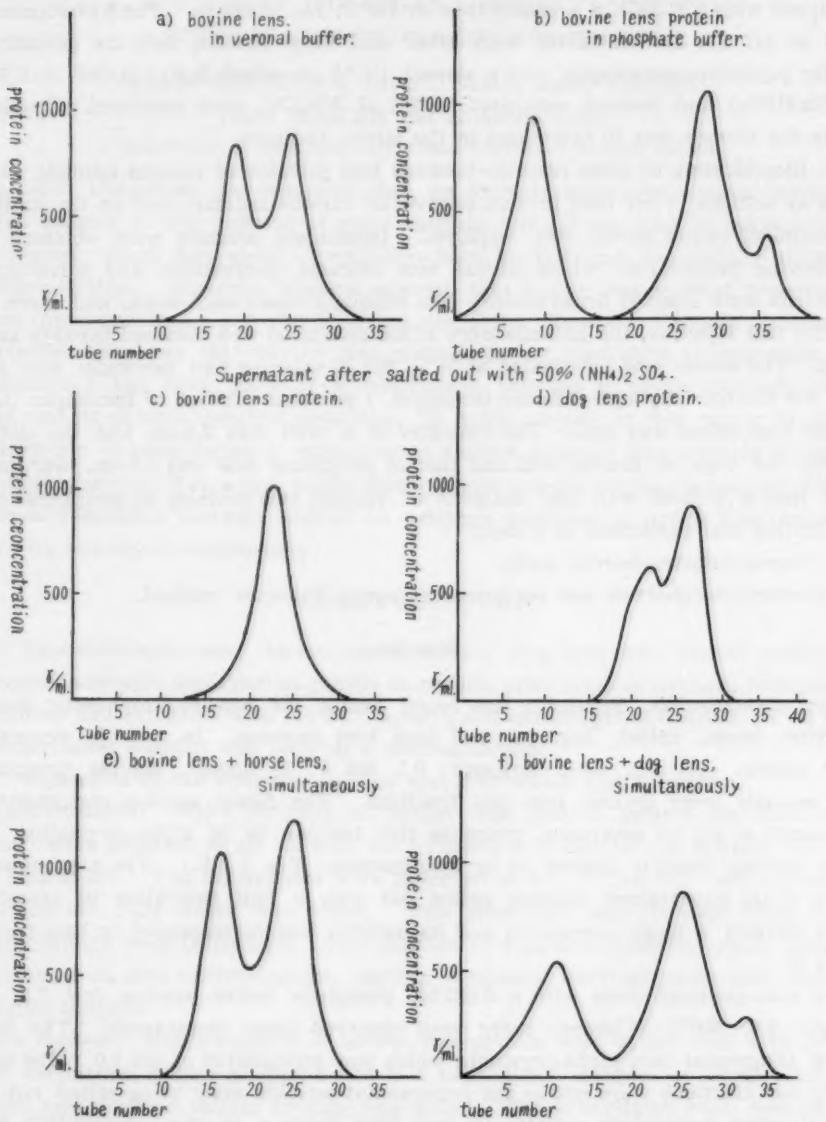
Results

Zone electrophoresis. Similarity was found among the zone-electrophoretic patterns of bovine, horse, rabbit, dog, cat and frog lens proteins. In a 0.1 M veronal-HCl buffer system (pH 8.6, ionic strength: 0.1, 600 V. 18 hours), soluble proteins of these animals were divided into two fractions. The faster moving component was precipitated at pH 5.0 maximum, revealing this fraction to be alpha crystallin. The slower moving fraction proved to be beta-fraction. (Fig. 1 a.b.) The electrophoretic pattern of the supernatant solution salted out with a half saturation of ammonium sulfate showed a single component and its mobility was corresponded to beta fraction. (Fig. 1 b)

On zone-electrophoresis with a 0.025 M. phosphate buffer system (pH 7.6, ionic strength: 0.07, 800 V. 16 hours), there were observed three components. The fastest moving component was alpha-crystallin which was precipitated at pH 5.0. The slower moving two fractions were left in the supernatant solution after being salted out with half saturated ammonium sulfate, so that beta fraction in the veronal-HCl buffer seemed to be divided into two components in the phosphate buffer system. (Fig. 1 c.d)

On a simultaneous application of lens proteins of two animals, for example, dog and bovine, etc. on a starch column, the electrophoretic pattern was the same as in a single application case, revealing the same electrophoretic mobility. (Fig. 1 e.f.) The

Fig. 1. Zone-electrophoretic patterns.



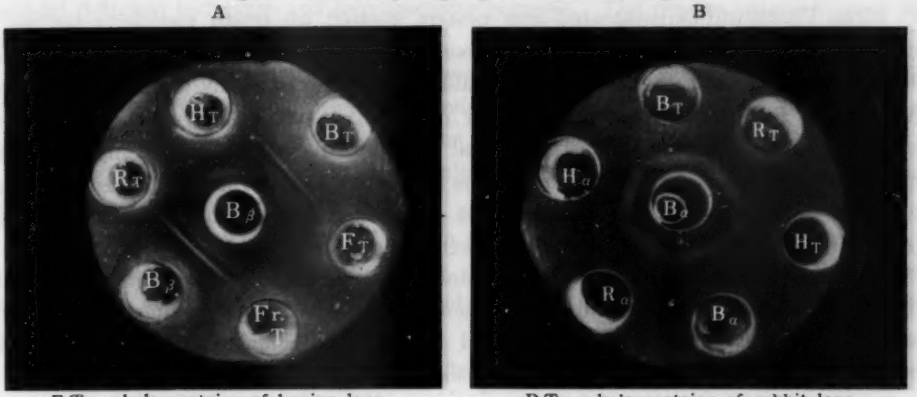
only difference was observed on human cataractous cases which almost showed disappearance of the first and the second component, demonstrating no decrease in third fraction.

Determination of N-terminal amino acid of alpha crystallin. Amino-terminal amino acid analysis was performed revealing the common results of the alpha-crystallins of

cow, horse and rabbit lens, glutamic acid.

Identification of cross reaction between lens proteins of various animals. On Ouchterlony's agar gel diffusion technique, the alpha-crystallin of bovine lens showed only a single antigenicity and cross reaction to alpha-crystallin of horse and rabbit lens protein. The beta-fraction of bovine lens protein showed three antigenicities, one of which was common to the beta-fraction of rabbit and horse lens protein. (Fig. 2)

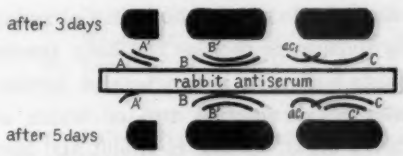
Fig. 2. Ouchterlony's agar gel diffusion technique,



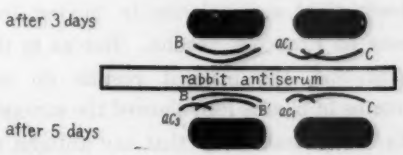
B T : whole proteins of bovine lens.
H T : whole proteins of horse lens.
FrT: whole proteins of frog lens.
R T : whole proteins of rabbit lens.
F T : whole proteins of fish lens.
Bα: bovine α-crystallin.
Hα: horse α-crystallin, Rα: rabbit α-crystallin, Bβ: bovine β-fraction
central well: antibody, peripheral well: antigen

Fig. 3. Immunoelectrophoresis.
a) whole protein of bovine lens

b) nucleus protein of bovine lens



c) cortex protein of bovine lens



Immunoelectrophoretic study. Bovine lens proteins possessed at least eight antigenicities which were represented by A-, B-, C-, A′-, B′-, C′-, αc_1- and αc_2-line of precipitation reaction. (Fig. 3a)

The nucleus proteins of bovine lens at least seven antigens (A-, B-, C-, A′-, B′-, C′- and αc_1) and the cortex proteins at least 5 (B-, C-, A′- αc_1- and αc_3-line). (Fig 3. b,c)

The antigens of alpha crystallin were represented by C-, C′- and ac_1-line, those of beta-crystallin by B-and B′- line, and those of the third fraction by A- and A′-line. The ac_3-line showed no correspondence to the antigen in any of the three fractions.

The antigens represented by B-, B′-, C′- and ac_1-line were common to the cortex and nucleus of bovine lens. However, the antigens for A- and C-line were specific for the nucleus. It seemed likely that the antigen for A-line was included in the third fraction which intensified the antigenicity of nucleus protein.

Discussion

Since Uhlenhuth demonstrated lens protein showed an organ specificity but not a species specificity, many investigators have studied on this problem. Hektoen and Schulhof reported α-crystallin and β-crystallin had immunologically distinct characters. Woods and Burky succeeded in isolating α-, β- and γ-crystallin which were immunologically pure. They demonstrated that α-crystallin possessed a complete organ specificity for homologous and heterologous animals. Beta-crystallin possessed an incomplete organ specificity for heterologous animals and tended to neutralize the antigenicity of α-crystallin; γ-crystallin showed the only antigenicity in vitro.
Recently newly developed excellent immunologic techniques have made it possible to study more clearily antigen-antibody reaction. Rao reported that bovine lens proteins had six antigenicities. Halbert succeeded in producing homologous lens antibodies in rabbit, evidencing that these antibodies showed five antigenicities on Ouchterlony's technique. He also stated that human cataractous lens and rabbit, frog, menhaden lenses had both organ and species specificity. François demonstrated that there at least eight antigenicities in bovine lens on an immunoelectrophoresis. However, no relationship does not seem to have been established organ specificity and its physicochemical properties.

On zone-electrophoresis, no differences were observed in water soluble proteins of various animal's lenses, such as bovine, horse, cat, dog and rabbit. N-terminal amino acid of α-crystallin of bovine, horse and rabbit lens is found to be glutamic acid. Furthermore, a purified bovine α-crystallin possessed only a single antigenicity which shows reaction to α-crystallin of horse and rabbit lens. From the present results, it is assumed that the organ specificity of lens protein is due to similarity of physical and chemical properties. However the β-fraction is divided into two fractions in a phosphate buffer on electrophoresis. As to the cause of this division, no decision has been reached whether β-fraction is dissociated in the phosphate buffer, or is denaturated during a zone-electrophoresis. These are three antigenicities in this fraction, two of which are species specific to bovine lens, one of which being organ specific to rabbit and horse β-fraction.

On an immunoelectrophoresis, there are at least eight antigenicities in bovine lens and the number of precipitation lines is the same as François' results. But as to the appearance of C′- and ac_3-line and lacking of ac_2-line, the present results do not necessarily coincide with his results. Nucleus proteins in bovine lens showed the stronger antigenic properties than those of cortex protein. It seems likely that the antigen of

A-line which is specific for nucleus, intensifies the antigenicity of nucleus protein.

Summary

Studies were made of an organ specificity and an antigenic structure of bovine lens protein on zone-electrophoresis, N-termial amino acid analysis, Ouchterlony's agar-gel diffusion technique and immunoelectrophoresis. The results obtained were as follows:

1. When zone-electrophoresis was performed on a pH 8.6 veronal buffer and a pH 7.6 phosphate buffer, soluble protein of bovine, horse, cat, dog and frog lens showed a similar electrophoretic pattern, revealing the same electrophoretic mobility.

2. N-terminal amino acid of α-crystallin of bovine, horse and rabbit lens protein was commonly found to be glutamic acid.

3. On Ouchterlony's agar gel diffusion technique, bovine α-crystallin possessed a single antigenicity which was common to α-crystallin of horse and rabbit lens. The beta fraction of bovine lens had three antigenicities, one of which was organ specific to the beta-fraction of horse and rabbit.

4. It is suggested that the organ specificity of lens protein was due to similarity of chemical and physical properties.

5. On an immunoelectrophoresis, bovine lens showed at least eight antigenicities, nucleus seven and the cortex five. The antigens represented by B-, B′- and ac_1-line are common to cortex and nucleus. However, the antigens represented by A- and C-line was specific to lens nucleus. It seemed likely that the antigen for A-line, which was included in the third fraction, intensified the antigen for A-line, which was included in the third fraction, intensified the antigenicity of nucleus protein.

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STUDIES ON NIGHT MYOPIA
REFRACTIVE CHANGES IN DIM ILLUMINATION

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Introduction

Night mypoia is one of the manifestations of night vision, and differs from the so-called myopia which is a phenomenon of day light vision. Not enough attention has been paid to this problem compared with other ametropia so far, and only a few reports have been presented in Japan.

Emphasizing the significance of night myopia in modern life, the author discussed in the previous report on the refractive changes of human eyes in relatively dim illumination (100～2.0 lx) and stated that spherical aberration and chromatic aberration played an important role in the etiology of night myopia.

Since night myopia is most remarkably observed in the environment with dimer illumination than those mentinned above, experiments under lower brightness levels are considered to be necessary for investigation of night myopia. In this report, results from the experiments in the low brightness levels and in telescope setting are presented with discussion.

Methods

1) The healthy males and females in the age range between 21-28 who had normal visual acuity were selected. The experiments were performed after dark adaption had been completed by keeping the individuals in a complete dark room for 30 minutes.

The apparatus was modified the Nagel's adaptometer and placed on a desk. A white tungsten lamp was used for the source of light. The size of a square illuminated window was 20×20 cm. The test target brightness levels were changed in 7 grades from $2.0\text{-}25\times10^{-4}$ rlx. by using natural filters and iris diaphragm.

The test target was a circular pattern of 10 cm. in diameter consisted of alternate black and transparent bars of equal width. That with pattern width of 1.5 cm. was regarded as 0.1 test type, and accordingly various test targets up to 0.5 test type were made.

Measurement of visual acuity was performed on the individual sitting on a seat 5 m. away from the test target and having his head and chin fixed on a holder. The so-called "concave lens effect" (CLE) was determined by the weakest concave lens which gave the highest visual acuity under the dim illumination.

Then, five experienced individuals (S.Y.: a 25-year male, T.M.: 24-year female, F.S.: 20-year female, H.A.: 28-year male, and K.H.: 26-year male) were selected out of 10 tested, and the CLE at 8 grades of brightness levels (7 grades mentioned above

plus 0.5 rlx.) were determined.

In other experiments, artificial circular pupil of 2 mm. in diameter was placed before the eyes of the tested individuals. Also, the CLE at 0.05, 0.005 and 0.0025 rlx. were measured by using interference filters A (λ max 496 mμ, T max 33.5%, $\Delta\lambda$ 1/2 9 mμ), B (λ max 567 mμ, T max 32.8%, $\Delta\lambda$ 1/2 8 mμ) and C (λ max 650 mμ, T max 30.5%, $\Delta\lambda$ 1/2 11 mμ).

2) Pupillary diameter was measured in three individuals (S.Y., H.A., and K.H.) who were watching test target at 2.0, 0.05 and 0 0025 rlx. at the distance of 5 m. In order to avoid errors in adaptation to each brightness level, photograph of the pupil with strobe-flash lamp was taken every 20 minutes. Based on the results, three kinds of artificial annular pupils of 4.0, 6.0 and 8.0 mm in outer diameter were made and the CLE was measured by applying these artificial pupils.

3) The CLE was measured 3 hours after the application of 1% atropine 2 drops 3 times every 3 minutes. The similar measurements were performed by using circular pupils and telescope setting. The telescope setting was made by attaching spherical convex lens (+1.0 dptr.) to object lens of binocular "Mikron" (6×30°) and placed 1m. away from the test target.

4) By the use of specially-made photographic device attached to Kajiura's slit-lamp

Table I. "Concave Lens Effect" in Dim illumination (Night Myopia)

rlx		S Y	T M	F S	H A	K H	average	AVERAGE
2		−0.75	−0.50	−0.25	−0.75	−0.50	−0.55	−0.600
1		−0.75	−0.75	−0.75	−1.50	−0.50	−0.85	−0.825
0.5		−0.75	−1.25	−1.00	−1.75	−0.75	−1.10	−1.025
0.1	R	−1.25	−1.50	−1.25	−1.75	−1.00	−1.35	−1.150
0.05		−1.25	−1.50	−1.25	−1.75	−1.25	−1.40	−1.425
0.005		−1.50	−0.75	−0.75	−1.25	−1.25	−1.10	−1.150
0.0025		−1.00	−0.50	−0.75	−1.25	−1.00	−0.90	−0.875
0.00025		−1.00	0	−0.50	0	−0.75	−0.45	−0.300
average		−1.031	−0.843	−0.812	−1.250	−0.875	−0.962	
2		−0.75	−0.50	−0.50	−1.00	−0.50	−0.65	−0.600
1		−0.75	−0.75	−0.75	−1.25	−0.50	−0.80	−0.825
0.5		−0.75	−1.50	−0.75	−1.25	−0.50	−0.95	−1.025
0.1	L	−0.75	−1.00	−1.00	−1.50	−0.50	−0.95	−1.150
0.05		−1.75	−1.50	−1.50	−1.75	−0.75	−1.45	−1.425
0.005		−1.75	−1.25	−0.75	−1.50	−0.75	−1.20	−1.150
0.0025		−1.00	−0.75	−0.50	−1.00	−1.00	−0.85	−0.875
0.00025		0	0	−0.25	0	−0.50	−0.15	−0.300
average		−0.937	−0.906	−0.750	−1.156	−0.625	−0.875	
AVERAGE		−0.984	−0.875	−0.781	−1.203	−0.750		−0.918

average: each eye AVERAGE: both eyes

Table Ⅱ. CLE by Use of the Annural Pupils

ap: annular pupil

rlx		SY			FS			HA			KH		
		2 mm ap	4 mm ap	6 mm ap	2 mm ap	4 mm ap	6 mm ap	2 mm ap	4 mm ap	6 mm pa	2 mm ap	4 mm ap	6 mm ap
2		−0.50	−1.00	−1.25	−0.50	−0.75	−1.00	−1.00	−1.25	−1.50	−0.50	−0.75	−1.00
1		−0.75	−1.00	−1.50	−0.50	−0.75	−1.00	−1.25	−1.25	−1.75	−1.00	−1.25	−1.25
0.5		−1.00	−1.25	−1.50	−0.75	−0.75	−1.25	−1.50	−1.50	−1.75	−0.75	−1.25	−1.50
0.1	R	−1.25	−1.50	−1.75	−0.75	−1.25	−1.50	−1.25	−1.50	−1.00	−1.00	−1.25	−1.50
0.05		−1.25	−1.50	−1.50	−0.75	−1.00	−1.50	−1.25	−1.75	−2.00	−1.00	−1.25	−1.50
0.005		−1.25	−1.25	−1.50	−0.75	−1.00	−1.50	−1.25	−1.50	−1.75	−0.75	−1.25	−1.50
0.0025		−1.00	−1.25	−1.25	−0.50	−0.50	−1.00	−0.75	−1.50	−1.50	−0.75	−0.75	−1.25
average		−1.00	−1.25	−1.43	−0.67	−0.85	−1.26	−1.25	−1.25	−1.60	−0.82	−1.10	−1.35
2		−0.75	−1.00	−1.25	−0.75	−0.75	−1.00	−1.00	−1.25	−1.50	−0.75	−1.00	−1.25
1		−1.00	−1.00	−1.25	−0.75	−1.00	−1.25	−1.25	−1.50	−1.75	−0.75	−1.25	−1.50
0.5		−1.00	−1.25	−1.50	−0.75	−1.25	−1.75	−1.50	−1.75	−2.00	−0.75	−1.00	−1.75
0.1	L	−1.25	−1.50	−1.75	−1.00	−1.25	−1.75	−1.50	−1.75	−2.00	−0.75	−1.25	−1.75
0.05		−1.50	−1.50	−1.75	−1.25	−1.25	−1.25	−1.75	−1.75	−2.00	−0.75	−1.00	−1.75
0.005		−1.25	−1.50	−1.50	−0.75	−1.00	−1.00	−1.25	−1.50	−1.75	−0.75	−1.25	−1.50
0.0025		−1.00	−1.25	−1.25	−0.50	−0.50	−1.00	−0.75	−1.50	−1.50	− 0	0	−1.50
average		−1.10	−1.28	−1.50	−0.75	−1.00	−1.28	−1.28	−1.53	−1.75	−0.64	−1.25	−1.50
AVERAGE		−1.053	−1.27	−1.38	−0.75	−0.93	−1.16	−1.27	−1.50	−1.68	−0.73	−1.04	−1.46

microscope, the depth of the eyechamber was measured at 2.0 rlx. and 0.005 rlx. while the persons tested watching test target. A 20-minute interval was taken between each photographic measurement. The position of the eye was kept unchanged during this time and the angle between the slit-lamp and camera was maintained at 45°.

Results

Magnitude of CLE in Night Myopia. According to the experiments in 20 eyes of 10 persons, the CLE was not observed at 1000 lx. At 2 rlx. of brightness, 80% of the eyes showed a shift to the myopic side and 40% of the eyes had the CLE of−0.5 dptr. At 0.05 rlx, 100% of the eyes showed the shift and the CLE ranged from−175 dptr. at maximum to−0.5 dptr. at minimum. However, at the brightness levels lower than 0.0025 rlx., the CLE tended to decrease on the contrary.

The table I shows the CLE in 10 eyes of 5 experienced persons. The decrese in visual acuity by over-correction was remarkable at the relatively low brightness levels, but it showed not much fluctuations below 0.05 rlx.

The maximum CLE was observed at 0.05 rlx. with average values of−0.96 dptr. in the right eye and−0.87 dptr. in the left eye. The individual difference was large with the range between−0.25 and−1.75 dptr.

1) Effect of Artificial Pupils and Change of Pupillary Diameter in Dim Illumination

Pupillary diameters were measured by vernier callipers on the negative films which were photographied with high luminous strobe-flash lamp. The pupillary diameters of S.Y., H.A. and K.H. were 6.4 mm., 6.5 mm. and 6.3 mm. (average 6.4 mm.) at 2 rlx., 6.9 mm, 7.0 mm and 7.0 mm (average 6.98 mm) at 0.05 rlx., and 7.4 mm, 7.5 mm and 7.6 mm (average 7.5 mm) at 0.0025 rlx. respectively.

It is needless to say that a human eye is a kind of optical instrument, and the degree of spherical aberration is proportional to the diameter of lens or maximum pupillary diameter in such an optical instrument. If the spherical aberration is an important factor in night myopia, pupillary diameter and the degree of night myopia should change in parallel relation. Accordingly, the CLE were measured by applying artificial annular pupils of 2 mm, 3 mm and 4 mm in diameter at various brightness levels. As shown in table II, the CLE increased with the increase in the annular diameter, and changes at each brightness level were similar to those in night myopia.

In experiments using circular pupil of 1.0 mm. in radius in order to know the effect of spherical aberration, depression of the CLE by 0.25 dptr.—0.75 dptr. was found at 0.05 rlx., at which the normal individuals showed the maximum CLE, and by 0.315 dptr. on an average at each brightness level.

Effect of Interference Filters. The Purkinje shift is said to be a shift around D and b Fraunhofer line of solar spectrum, and the chromatic aberration between the shift and the refractive system of the eye is considered to have some relation with night myopia. Nakagawa, J. (1950) reported a shift of 0.4 dptr. between D and E-Fraunhofer line. Three kinds of interference filters were used in my experiment but the filter B (of wavelength 567 mμ) appeared to be most significant in relation with chromatic aberration for the above-mentioned reason. The depression of the CLE caused cy this filter was 0.225 dptr. on an average of 10 eyes as shown in table III. The CLE increased by 0.26 dptr. by the use of filter A, and decreased by 0.54 dptr. by the use of filter C.

Effect of Atropine. The tested individual H.A. showed the CLE of—1.75 dptr. at 0.05 rlx, but it was depressed by 1.0 dptr. by application of atropine and by 1.25 dptr. by the additional use of circular pupil. (Fig. III) The average value of depression at each brightness level was 0.719 dptr. in the atropinized eye, and 1.03 dptr. in the eye with atropine+circular pupil. In K.H., the depression of the CLE was 0.25 dptr. with atropine and 0.5 dptr. with atropine+circular pupil at 0.05 rlx, and 0.25 and 0.5 dptr. respectively at each brightess level. In S.Y., the CLE was not observed with atropine but +0.75 dptr. at 500 lx.

2) Measurements on Telescope Settings and Change of Eye-chamber Depth in Dim Illumination

Measurements on telescope setting revealed the CLE -0.2 dptr. in the right eye and —0.47 dptr. in the left eye in the case S.Y., and—1.05 and—1.25 respectively in the case K.H.. Thus the CLE showed a shift to the myopic side which is considered to be a

Table I. Depression of the CLE

1) circular pupil r=1.0 mm ((—): increase of CLE (+): decrease of CLE)

	S	Y	H	A	K	H	T	M	F	S
rlx	R	L	R	L	R	L	R	L	R	L
0.05	+0.50	+0.50	+0.75	+0.50	+0.50	+0.25	+0.75	+0.75	+0.50	+0.50
0.005	+0.50	+0.50	+0.50	+0.25	+0.50	+0.25	+0.25	+0.50	+0.25	+0.25
0.0025	+0.25	+0.25	+0.50	+0.25	+0.50	+0.50	+0.25	+0.75	+0.25	+0.25
average	+0.416	+0.416	+0.583	+0.333	+0.500	+0.333	+0.416	+0.666	+0.333	+0.333
AVERAGE	+0.416		+0.458		+0.416		+0.541		+0.333	

2) interference filter B 567 mμ

	S	Y	H	A	K	H	T	M	F	S
rlx	R	L	R	L	R	L	R	L	R	L
0.05	+0.25	+0.50	+0.75	+0.25	+0.25	+0.25	+0.25	+0.25	+0.50	+0.25
0.005	+0.50	+0.50	−0.50	+0.25	−0.50	+0.25	+0.25	+0.25	+0.25	−0.25
0.0025	+0.25	+ 0	+0.25	0	+0.50	+0.25	+0.25	+0.50	+0.25	0
average	+0.333	+0.333	+0.166	+0.166	+0.083	+0.250	+0.250	+0.333	+0.333	0
AVERAGE	+0.333		+0.166		+0.166		+0.291		+0.166	

3) atropine

rlx	S Y	H A	K H	
0.05	+1.75	+1.00	+0.25	+1.000
0.005	+1.75	+1.00	0	+0.916
0.0025	+1.00	+0.50	+0.25	+0.583
average	+1.500	+0.833	+0.166	+0.833

1)′

rlx	R	L	average
0.05	+.060	+0.50	+0.550
0.005	+0.40	+0.35	+0.375
0.0025	+0.35	+0.40	+0.375
average	+0.449	+0.416	
AVERAGE			+0.432

4) atropine+circular pupil

rlx	S Y	H A	K H	
0.05	+1.75	+1.25	+0.50	+1.166
0.005	+1.75	+1.25	+0.25	+1.083
0.0025	+1.00	+1.00	+1.00	+1.000
average	+1.500	+1.166	+0.583	+1.083

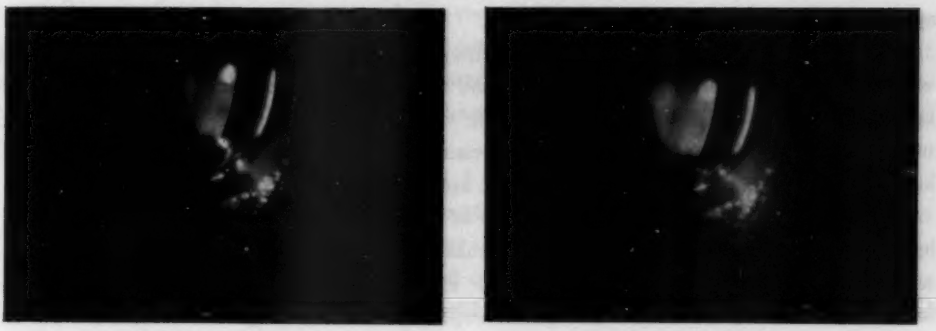
2)′

rlx	R	L	average
0.05	+0.40	+0.50	+0.350
0.005	0	+0.20	+0.100
0.0025	+0.30	+0.150	+0.225
average	+0.233	+0.216	
AVERAGE			+0.225

contrast (normal eyes)

rlx		S Y	T M	F S	H A	K H	average	AVERAGE
0.05	R	−1.25	−1.50	−1.25	−1.75	−1.25	−1.40	−1.425
	L	−1.75	−1.50	−1.50	−1.75	−0.75	−1.45	
0.005	R	−1.50	−0.75	−0.75	−1.25	−1.25	−1.10	−1.150
	L	−1.75	−1.25	−0.75	−1.50	−0.75	−1.20	
0.0025	R	−1.00	−0.50	−0.75	−1.25	−1.00	−0.90	−0.875
	L	−1.00	−0.75	−0.57	−1.00	−1.00	−0.85	
average	R	−1.250	−0.916	−0.916	−1.416	−1.166	−1.132	
	L	−1.500	−1.166	−0.916	−1.416	−0.833	−1.166	
AVERAGE		−1.375	−1.041	−0.916	−1.416	−1.000		−1.149

Fig. 1. Change of eye chamber-depth.



S.Y. left eye (2.0 rlx) S.Y. left eye (0.05 rlx)

phenomenon known as "instrument myopia". The CLE was maximum at 0.05～0.0025 rlx. and tended to decrease at extreme low brightness levels. These tendencies were similarly seen in other experiments without telescope setting. The CLE measured on telescope setting shifted further to the myopic side compared with the CLE measured using spherical lens. For example, the average CLE on telescope setting in 10 eyes were−1.256 dptr in the right eye,−1.378 dptr. in the left eye and−1.307 dptr. in the both eyes. The CLE decreased considerably in the atropinized eyes (by 0.25～1.75 dptr.)

The individual difference in the CLE among 3 persons at each brightness levels was in the range of 1.0～1.5 dptr. in the non-atropinized eyes, but decreased to 0～05 dptr. in the atropinized eyes.

Fig. 1 is a photography of the left eye of the case S.Y. by slit lamp. As one sees, the eye-chamber depth decreased when the brightness levels was changed from 2 rlx. to 0.005 rlx. in S.Y., as well as in H.A. and K.H.

Discussion

The relation between night myopia and illumination. Night myopia is known to appear at the brightness level of twilight (several lux.) and increase in the severity with further lowering in the environmental illlumination. We observed in the previous experiments the appearance of the CLE already at the level of 70 lx. In general, clear vision is frequently obtained by applying a mild concave lens of−0.25 dptr. in emmetropia with normal visual acuity. The early appearance of CLE observed in the previous experiments may be due to the errors based on this phenomenon since clear vision in inexperienced individuals was used for determination of the CLE. (It is interesting to note the early appearance of the CLE at 100～50 lx. in patients with accomodative asthenia.) In fact, when clear vision was used for determining the CLE in night myopia at dim illumination, a shift of the CLE by 0.5 dptr. toward the myopic side was observed. The shift was larger in the telescope setting and in the range of 1.0～2.0 dptr. In the present experiments, 80% of the tested individuals showed the CLE at 2

rlx. The results from the previous and present experiments indicate that night myopia appears about 10 lx. of brightness.

The ratio of the uncorrected visual acuity to corrected visual acuity in night myopia was 1 : 1.1～1.6, and the maximum value was at around 0.5 rlx. Nakagawa, J. reported that the ratio was 1 : 1.5～2.0 with maximum value at 0.1 lx., and Cabell et al. 1 : 2 with maximum value at 10^{-3} ml. Decrease in visual acuity by over-correction was remarkable down to 0.5 rlx., but did not change appreciably below this brightness level.

According to Koomen et al. magnitude of night myopia reaches to the maximum level at 0.02 ml. Schober, H. stated that the magnitude increased progressively with the decrease in illumination. In our experiments, it reached to the maximum level at 0.05 rlx. Bowman and Brink reported that the magnitude decreased on the contrary in extreme dim illumination. Similarly in our experiments, it decreased at 0.0025 rlx., and the CLE was not observed in 4 eyes of 2 cases (T.M. and H.A.) and in the left eye of a case (S.Y.) at 0.00025 rlx. In general the determinaton of the CLE is difficult in such extreme dim illusion because of poor recognition of the test targets by tested persons. Therefore we used specially designed test types instead of ordinary ones. Nakajima and Kojima stated that the test types such as Landolt's ring are not suitable for use in dim illumination because of jumping or distortion of image, and recommended a test type of U-form with width of 1/5 of diameter. Nakagawa recommended -form test target. The pattern targets used by us were suitable for measurements in dim illumination and proved to be reliable by comparison with Inoue's test type.

Effect of spherical aberration. When artificial circula pupil is used, problems such as diffraction, focal length or magnitude of incident light must be taken into consideration. Most of the spherical aberration was considered to be removed by application of circular pupil since diameter of the lens was made small by it. The effect of spherical aberration was 0.315 dptr. on an average. The fact that no CLE was observed in 9 eyes of 5 individuals (T.M., F.S., H.A. and K.H. and the left eye of S.Y.) at the lowest illumination level of 25×10^{-4} rlx., may be explained by the decrease in visual acuity due to decreased magnitude of incident light and difficulties in its determination, rather than by the decreased spherical aberration with pin-hole glass.

The presence of individual difference in the degree of night myopia, shown by the difference of 0.25～0.75 dptr. at 0.05 rlx., may be an interesting point.

Changes in the pupillary diameter in dim illumination is another problem. Du-Bois Reymond succeeded in photographing the pupil by magnesium flach in 1888. In dark environment, the pupil shows mydriasis immediately by dark adaptation and reaches to a completely balanced state sometime later. The time required for the pupil to reach the balanced state was reported by Raurens (1928) to be 6 minutes and by Garten (1897) and Weiler (1910) to be 15 minutes. Recently Shimazaki (1958) measured the pupillary diameter by using infra-red image converter (image tube 1925) and obtained

an average value of 7.4 mm. (5.6~8.1 mm.) in 8 individuals with age between 20~30 years 20 minutes after going into dark room. The pupillary diameters reported by Du-Bois Reymond and Weiler were 10 mm. and 7.4 mm. respectively. It appears that the pupillary diameter at the completely balanced state is about 7~8 mm. The diameter at dim illumination was reported by Hayakawa to be 6.7 mm. at 0.1 lx. and 7.12 mm. at 0.01 lx.

The pupillary diameter was 6.4 mm. at 2 rlx., 6.98 mm. at 0.05 rlx. and 7.5 mm. at 0.0025 rlx. in our experiment. Based on the results, annular pupils were made and the CLE were measured. This maneuver required an extreme skill. It was very difficult to put test target on the fovea, and ocular movement similar to nystagmus was frequently encountered. However, the results indicated the CLE caused by various annular pupils showed a tendency similar to that in night myopia.

The CLE was considereably depressed by application of atropine to the eye. Koomen et al explained the effect of homatropine in night myopia by diminished spherical aberration, but it seems that more complicated factors are involved in this problem.

In telescope setting, the individual difference in the CLE among S.Y., H.A. and K.H. was 1.0~1.5 dptr. in normal eyes and 0.25~0.5 dptr. in atropinized eyes at each illumination level. This decrease in individual difference suggests that the problem of "Akkommodationsruhelage" described below may be involved as well as the decrease in spherical aberration.

The effect of spherical aberration is supposed to be 0.315 dptr. on an average, or around 0.5 dptr. at most. Therefore, spherical aberration alone can not explain the fluctuations in night myopia ranging -1.0~1.75 dptr.

Effect of chromatic aberration. Although the Purkinje shift and refractive system in chromatic aberration have been paid much attention lately as to the etiology of night myopia, chromatic aberration is considered to be not so significant as expected. As described above, it could not cause depression of the CLE more than 0.3~0.4 dptr. at most. The interference filter used in the experiment has a merit that it gives only the light with wanted wavelength, differing from the ordinary filters. The monochromatic light from spectrum seems to be most suitable, but a certain level of dim illumination can not obtained so easily. Accordingly, the use of interference filter may be justified in these experiments. Chromatic aberration in night myopia measured by us was 0.225 dptr. on an average.

Effect of accommodation. There have been two sorts of ideas as to the main etiologic factor of night myopia. These represented by Koomen et al think the changes in spherical aberration plays the main role, and others headed by Otero consider the changes in accommodative function for that.

J.M. Otero thought that night myopia was closely related with the changes in accommodative function of the eye, and stated that night myopia is essentially night presbyopia in which the lens takes the resting position (1.25 dptr.) in dark place. The

night presbyopia was discovered by Duran and Palacios (1943) independently and later confirmed by Wald & Griffin, Schober, and Kühl.

Steiger and Betsch introduced an idea of "Akkommodations-ruhelage" by observing the difference of 1.5 dptr. between degree of normal eye refraction (+0.5 dptr.) and its value measured by the use of eye-piece control method (-1.0 dptr.).

Siebeck measured accommodation in 10 individuals of 25-30 years of age, obtaining the value of 0.63 dptr. which was lower than far point, and claimed that it was the "Akkommodationsruhelage". Lau and Mütz also recognized the presence of it.

We measvred the eye-chamber depth at dim illumination, and found that the depth decreased by changing the illumination level from 2 rlx to 0.005 rlx. However, this decrease should not be related immediately with the changes in accommodation. It is not known at present whether this decrease in depth is caused by advancement of the lens due to mydriasis, by Akkommodationsruhelage, or by merely individual difference.

The problem of accommodation appears to be important and further investingations are required.

Summary

In previous report, the author mode mention of night myopia has important association to various states in modern life, then demonstrated the presence of night myopia experimentally and to investigate the mechanism of it. In this report, the author gave proof of night myopia in dim light and investigated the causes of it again.

Subjects were 20 eyes of emmetropia. Changing the grade of brightness to 6 grades from 2 rlx to 25×10^{-5} rlx, refraction of eye was measured. The similar experiments with 5 experienced test persons were carried out and at same time it were repeatedly observed the influenc of the application of annural artificial pupils and interference filters before subject's eye on the night myopia and then the influence with atropine paralyzed eye upon night myopia.

The focussing properties of human eye becomes relatively nearsighted in dim light (night myopia). It begins to at about 10 lux and gradually it grew larger as the brightness was further reduced and it attained to a maximum value in about 5×10^{-2} lux. Individual difference of degree of night myopia was very remarkable from 0 to—1.75 dptr. (average-0.918 dptr.) but on the contrary the night myopia seemed to decrease at extreme low brightness. It is proved that night myopia in majority was due to spherical aberration, accommdation and partly due to chromatic aberration.

Especially, accommodation was very significatly related to night presbyopia, rest position of the lens and instrument myopia.

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STUDIES ON HERPES CORNEAE
REPORT 1. BIOMICROSCOPIC AND HISTOLOGIC FINDINGS ON THE EXPERIMENTAL HERPETIC KERATITIS

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Preamble

Recently in Japan, herpetic keratitis which was reported by Dr. Thygeson as a kind of most common keratitis in U.S.A. has rapidly increased also here within several years and the aspect of the disease has become serious. This tendency is noticed with deep interest. In the present report, biomicroscopic and histologic findings on the course of the experimental herpetic keratitis of the rabbits were traced and mutuality between both findings was investigated.

Method of Experiments

Corneal scrapings obtained from the lesions of the patients affected with herpetic keratitis were inoculated into the scarified cornea of the rabbits and typical dendritic keratitis was produced in 7 out of 11 cases. Afterwards, serial passage was performed through the cornea of the rabbits. On the other hand, fixation in the brain of the mice was tried and 20% emulsion of the brain re-inoculated into the cornea of the rabbits. On total 50 cases of the experiment, biomicroscopic observation was performed every day. Meanwhile, the lesions were removed and investigated histologically, stained with H.E., Man-Masson and Azan.

Results of Experiments

From the findings of biomicroscopy, the entire course of experimental herpetic keratitis was classified as follows.

Incubation
The 1st stadium of small infiltration
The 2nd stadium of dendritic ulceration

The 3rd stadium of diffuse opacification

The 4th stadium of decline

1. Incubation

In this stadium, biomicroscopy does not detect any significant changes. However, histologically, slight proliferation of superficial epithelial cells, intra and inter-cellular oedema and swelling of the nuclei were observed near the scratchings.

2. Stadium of small infiltration

Biomicroscopic findings. After 16 hours—7 days (averge 1.86 days) from the inoculation small infiltrative focuses which are composed of white turbid spots comparatively transparent, and fine vesicle-like protuberances and fluorescein-test positive small punctate parts are produced, mainly along the scratch marks.

After 2-7 days (average 3.08 days), these small infiltrative focuses increase and develop to a round type. After 2-12 days (average 4.79 days), these focuses are disseminated throughout of the cornea.

Histologic findings. Intercellular oedema and swelling of the nuclei gradually increase the intensity and exfoliation of the superficial layer of the epithelium is produced. These changes develop from the superficial layer to the middle layer of the epithelium.

Mitosis is especially noteable at the basic layer.

3. The 2nd stadium: Stadium of dendritic ulceration

Biomicroscopic findings. In small infiltrative focuses, fluorescein-test positive parts become star-shaped. Each process of the star elongates and has bead-like knobs at the head. This figure is observed after 2-7 days (average 3.64 days). After 3-9 days (average 4.85 days), the figure developes further, to typical dendritic feature. After 3-14 days (average 6.41 days), the pattern spreads throughout of the cornea.

The bottom of an irregular cord-like dendritic ulcer frequently reaches the upper layer of the parenchyma. On the epithelium adjacent to the ulcer, vesicle-like protuberances, fluorescein-test positive small spots and punctate opacity are together present, as same as the small infiltrative focuses. The surface of such epithelium is rough and slightly swells, surrounded by a halo-like diffuse opacity.

As growing in violence, from the epithelial layer around the ulcer to the upper layer of the parenchyma, densely opaque and fine spots gather massively and oedematous opacity reaches the middle layer of the parenchyma.

Iritis is also produced. After 6-12 days (average 7.75 days), blood vessels from upper part invade the surface of the cornea.

Histologic findings. On account of cell necrosis in the area of the ulcer the superficial epithelium is falling off. The arrangement of the cells is irregular.

The nuclei of epithelial cells swell so much that the size is 3-4 times the normal state, chromatin becomes granular and reticular, the figure takes on the so-called "Swiss cheese-like feature". Afterwards, they gradually gather at the margin of the nucleus and form a ring or crescent. This feature is the so-called "Balloon degenera-

tion". Occasionally, these nuclei take the course towards pyknosis and condense.

After 4 days (average), round eosinophile inclusion bodies, viz. Lipschütz's body, appear in the nuclei. The feature is observed in the epithelial layer which is intensely involved. At this stage, specific polynuclear epithelial giant cells, which have 2-15 nuclei are observed.

On the superficial layer of the parenchyma, infiltration of pseudoeosinophile leucocytes and lymphocytes along the fibers of the lamellae, oedema, blurred architecture of the lamellae are observed.

4. The 3rd stadium: Stadium of diffuse opacification

Biomicroscopic findings. After 6-16 days (average 7.42 days), milky diffuse opacity is produced and after 7-18 days (average 11.2 days) developes throughout the cornea with few traces of a dendritic feature. But frequently the limbus of the cornea is not involved and remains transparent. The parenchyma has oedematous opacification and become thick. A mass of grayish white spots of opacity is observed up to the middle parenchyma.

Histologic findings. The surface of the epithelium becomes completely rough. Every layer of the epithelium is distinctly detached, one from the other. Consequently, a wave-like space is formed everywhere. Occasionally, retention of fluid is observed in the space.

Epithelial cells have vacuolar degeneration and the nuclei condense. The feature of basal membrane is irregular. It disappears at some parts, while, it becomes thick at other parts and makes crépe-like ripples.

Intranuclear inclusion bodies and polynuclear epithelial giant cells are completely absent.

In the parenchyma, there are very intense oedema, degeneration and proliferation of fixed cells, and immigration of fibroblasts, leucocytes and lymphocytes. At the limbus, new vascularization is observed.

5. The 4th stadium: Stadium decline

Biomicroscopic findings. During from 7-18 days to 10-28 days after the inoculation, oedema and opacity in the epithelium and parenchyma are gradually improved. After 18.1 days (average) experimental herpetic keratitis of the rabbits heals spontaneously.

The surface becomes smooth, although the thickness of the epithelium is uneven. Partly, faint diffuse opacity remains, but it proves to be completely negative by fluorescein-test. Occasionally, several bullae are formed.

The opacity in the parenchyma is classified into 2 types. One is diffuse opacity which forms a triangle. It bases on the epithelial basal membrane and has an apex in the middle parenchyma. The other is lineal and dense opacity along the lamellae. Occasionally, 2 or 3 lines of lineal opacity run parallel to each other.

At this stage, blood flow in the invaded vessels becomes weak and iritis is also improved remarkably. However, even when the focus in the cornea has no inflammation,

capillaries on the iris and fine dusty turbidity in the anterior chamber are still observed.

Histologic findings. Especially in the middle epithelial layer, multiformed cells which have large nuclei and abundant protoplasma propagate to repair the defects. At the beginning, the arrangement of these cells is irregular and a squamous epithelial layer is absent. However, the arrangement gradually becomes regular and recovers to an almost normal state. Partly, the epithelium thickens by overpropagation of the cells; or in the basic layer, sevral cells show vacuolar degeneration.

In the epithelium, infiltration of lymphocytes and pseudoeosinophile-leucocytes is exhibited more or less on all cases. The basement membrane thickens and forms folds at several parts. In the parenchyma, blurred architecture of the lamellae due to necrosis, propagation of fixed cells and fibroblasts and interlamellar cell infiltration are present, even though no evidence of the inflammation exists clinically.

Conclusion and Discussion

Isolation of the virus. 7 cases among 11 cases of the patient had positive results on the isolation to the cornea of the rabbits. Especially, 4 cases on which dendritic keratitis was fresh and the active state showed positive results, on all occasions. Hanna, and Thygeson reported, that isolation of the virus from dendritic lesion with a high rate was successful. Histologically, it was revealed that intranuclear inclusion bodies which had close association to herpes simplex virus appeared only at this stage, in consequence of this fact it would be easy to isolate the virus at the stage of dendritic ulceration.

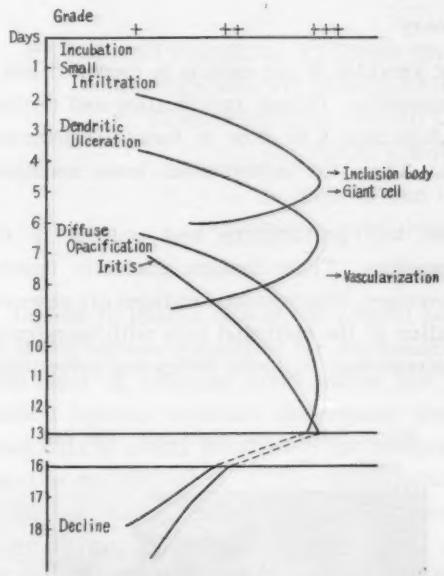
Material of the inoculation. It was discussed by many investigators that the pathogenic character, affinity for tissue and antigenosity of herepes simplex virus differed with each strain. In the present experiment, 3 kinds of the material were used. They were scrapings of corneal lesions of humans and rabbits and fixed antigen of the brain of mice. On any occasion, typical dendritic keratitis was produced. On the form of the focus and course of the disease, no remarkable difference was observed. Among the eyes of the rabbits on which the same material was inoculated by the same method, minor differences were observed in the term of incubation and the course of keratitis. Encephalitis was not produced on any occasion.

The course of herpetic keratitis of the rabbit. After the inoculation, it took incubation 16 hours to 7 days. Afterwards, the changes were perceived at first with the biomicroscope. Then, the course was classified into 4 stages. They were the 1st (small infiltration), the 2nd (dendritic ulceration), the 3rd (diffuse opacification) and the 4th (stadium of decline). Table 1 showed the needed period to produce the focuses of each stadium. The average course of 50 eyes of the experiments was shown on Table 2. Wolter etc. reported, that clouding developed on the cornea after 3-4 days, typical dendritic ulcer appeared on about 80% after 5-10 days and 90% healed after 18-20 days, leaving superficially vascularized scars. Compared with the author's experiments,

Table I. Period to Produce the Lesion of each Stage after Inoculation

Stadium \ Grade	+	++	+++
	Days		
1. Small Infiltration	0.7—7 Days 1.86 (average)	2—7 3.08	2—12 4.79
2. Dendritic Ulceration	2—7 3.64	3—9 4.85	3—14 6.41
3. Diffuse Opacification	4—14 6.45	6—16 7.42	7—18 11.29
Vascularization	4—11 7.75	13—18 16.00	

Table 2. Average Course of 50 Eyes



development of the lesions was slightly late, but the period to reach the decline assimilated very well.

Relation between biomicroscopic findings and histologic findings. After observation with the biomicroscope, the focuses were investigated histologically. As the results, it was assumed that the following relations were present between both findings.

1. Small vesicle-like protuberances and vesicles are produced by intra and intercellular oedema, detachment of each epithelial layer and accumulated fluid in between.

2. Small punctate opacities which are present in small infiltrative lesions or around the dendritic ulcer are produced by degeneration of the cells with nuclear degeneration. When degeneration is slight, it is perceived as oedematous opacity.

3. The parts which take on a deep colour by fluorescein-test are defective parts due to necrosis of the epithelium. At the environs of the focus, the rough surface with slight degeneration takes on diffuse and light colour by fluorescein.

4. Dense and grayish white opacities which are observed in the epithelium and parenchyma correspond to the infiltration of lymphocytes, pseudoeosinophile-leucocytes and fibroblasts, propagation and degeneration of stromal fixed cells.

5. Oedematous opacities which are present at the stage of diffuse opacification correspond to disarrangement and vacuolar degeneration of the epithelial cells and detachment of each layer of the epithelium.

6. Diffuse or lineal opacities in the parenchyma ase produced by oedema and degeneration of the corneal lamellae.

7. At the stage of decline, the surface of the epithelium becomes smooth and it is not stained with fluorescein. The reason for this is attributed to reconstruction of the epithelium. The defects are repaired by propagated cells and each epithelial layer is formed. Consequently, the architecture recovers to a normal state. The thickness of the epithelium uneven because propagation of the cells partly in excess, and partly deffective.

However, it is impossible to explain the genesis of all the opacities from a histological angle, because they are produced by various factors, such as the changes of chemical substance or micelle-architecture etc…

Summary

1. The course of experimental herpetic keratitis of the rabbits is classified into 4 stages, namely small infitration, dendritic ulceration, diffuse opacification and decline. On the average, it takes 1.86 days, 3.64 days and 6.45 days to form characteristic lesions in each stadium, from the inoculation. Then, the inflammation heals spontaneously after 18.1 days.

2. Histologically, swelling of the nuclei and proliferation and oedema in the epithelium are observed slightly, even in incubation. These changes gradually develop and increase the intensity. After 4-5 days (average), characteristic changes are observed on all cases. They are ballooning degeneration of the epithelial cells with marginated chromatin of the nuclei and appearance of intranuclear inclusion bodies and polynuclear epithelial giant cells.

Fig. 1. Inclusion bodies, ballooning degeneration in the epithelium. The 2nd stadium (Dendritic Ulceration).

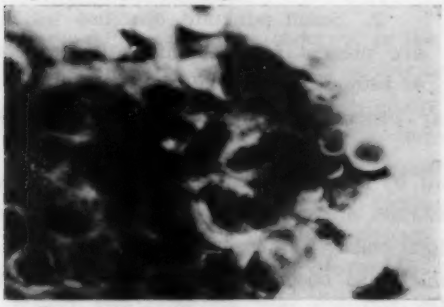


Fig. 2. Polynuclear giant cell. The 2nd stadium (Dendritic Ulceration).

Even after clinical recovery of the inflammation, abnormal proliferation and vacuolation of the epithelial cells, degeneration of basal membrane, cell infiltration in the

Fig. 3. Vacuolation in the epithelium, degeneration of the basal membrane and oedema in the parenchyma. The 3rd stadium (Diffuse Opacification).

parenchyma, degeneration of the lamellae and propagation of fixed cells are observed, histologically.

3. The investigation on mutuality between biomicroscopic and histologic findings was undertaken.

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PATHOHISTOLOGICAL STUDIES ON ARACHNOIDITIS, WITH ESPECIAL REFERENCE TO CIRCULATION DISTURBANCES OF THE CEREBROSPINAL FLUID INQUIRY INTO ETIOLOGY OF NEURITIS RERTOBULBARIS

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Introduction

In 1940 Jo Imachi, one of the present authors, mentioned chiasmal arachnoiditis as one of the factors responsible for development of neuritis retrobulbaris, and stated that many cases of chiasmal arachnoiditis had been cured by surgical intervention. In Imachi's opinion metabolic disturbance due to circulation disturbance of the cerebrospinal fluid is chiefly responsible for cerebro-optic nerve damage in arachnoiditis. With regard to the circulation of the cerebrospinal fluid, many points still remain unascertained, but Kafka, noting that some substances introduced into the blood stream selectively go over into the fluid, assumed the existence of a barrier between the blood and the fluid. As it is quite possible that abnormal permeability, secretion and absorption of the fluid may be present in cases of chiasmal arachnoiditis, specimens of the arachnoid taken at the time of craniotomy were pathohistologically examined. And also measurement of secretion and absorption time of the fluid, and permeability test by intravenous injection of uranin were conducted in order clinically to determine the existence of circulation disturbance of the fluid in these cases. The permeability test was further done with rabbits whose arachnoid had been artificially inflamed by injecting talcum powder into the cisterna magna. Clinical relationship between the cerebrospinal fluid and the optic nerve was thus experimentally investigated.

I. Pathohistological investigations of the arachnoid in various forms of cerebro-optic nerve damage

Specimens of the arachnoid membrane were prepared from 300 cases of chiasmal arachnoiditis who were craniotomized by Imachi for therapeutic purpose, and stained with hematoxylin-eosin, and Van Gieson's stains. Of these, 100 specimens which were complete in all the layers of the arachnoid were examined. The cases investigated were of the following diseases: neuritis retrobulbaris of unknown etiology; Leber's familial optic atrophy; optic atrophy (simple or postinflammatory of unknown etiology, traumatic or postarachnoiditis traumatica, syphilitic or tabetic, postmeningitis tuberculosa, and of chiasmal tumor origin); and meningeal affections accompanied by papilledema. These cases were placed into five divisions according to length of interval after onset of disorder (less than 1 month, 1–6 months, 1–3 years, and more than 3 years).

Less than one month after onset of disorder (6 Cases). Thickening and proliferation of endothelioid cells of the arachnoid were not noted, except in one case of Leber's disease. In this case were noted nuclear swelling of endothelioid cells, and chromatolysis of a moderate degree. Moreover, calcium deposits, too, were found among endothelioid cells. In Photo 2, these deposits were seen as deeply stained with hematoxylin, and surrounded by endothelioid cells.

Thickening and proliferation of the connective tissue were noted in almost all the case. Mild emigration of lymphocytes was also noted, but no trabeculae were found in the subarachnoid tissue. In two cases fibrin deposits were observed in the subarachnoid space. In these cases emigration of lymphocytes, too, was very extensive, and also moderate emigration of leucocytes was noted. (Photos 3 and 4)

One to 6 months after onset of disorder (15 cases). In most cases thickening and proliferation of endothelioid cells were rather mild, but in some cases these findings were moderately pronounced. (Photo 5) Mild emigration of lymphocytes was noted in 5 cases. In a case of retrobulbar neuritis three months after onset of disorder moderate emigration of lymphocytes was noted. Some leucocytes had also emigrated. (Photo 6)

Photo. 1. The normal arachnoid (the left side facing the dural membrane; the right side subarachnoid cavity).

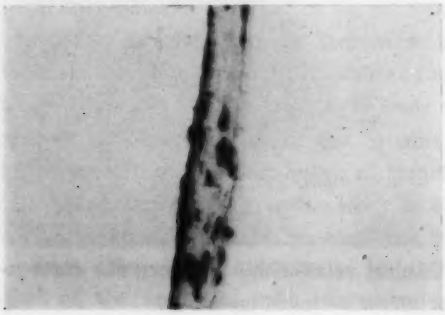


Photo. 2. Calcium deposits into endothelioid cell layer.

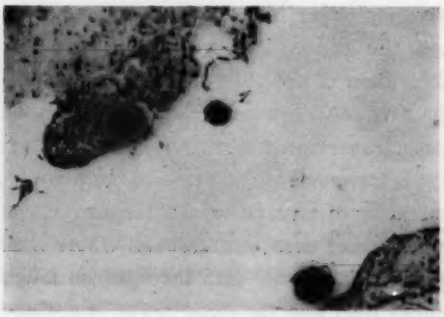


Photo. 3. Exudation of small round cells, and fibrin deposits.

Photo. 4. Fibrin deposits.

Photo. 5. Proliferation and thickening of endothelioid cell layer.

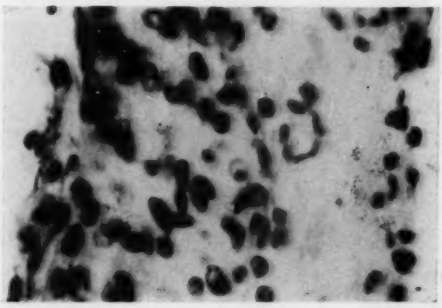


Photo. 6. Exudation of small round cells into endothelioid cell layer.

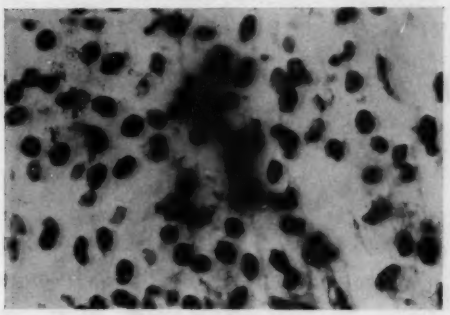


Photo. 7. Thickening of connective tissue layer.

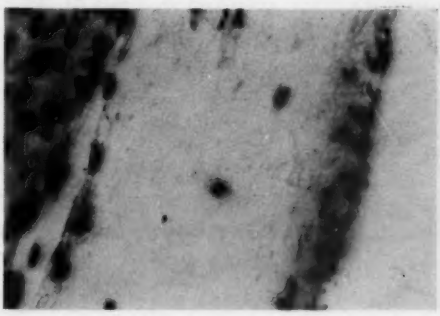


Photo. 8. Emigration of small round cells into connective tissue layer.

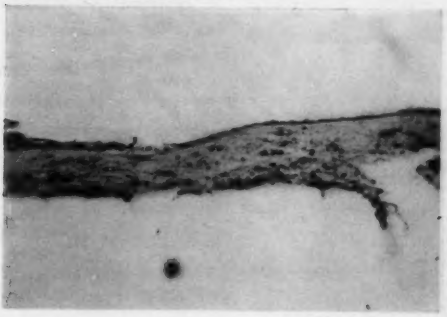


Photo. 9. Small round cells in subarachnoid tissue.

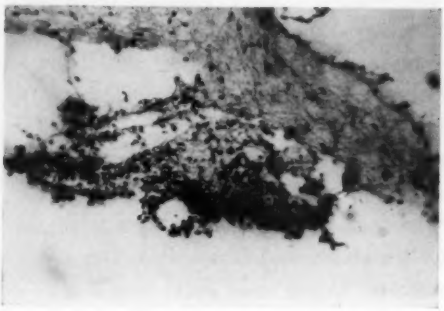


Photo. 10. Thickening of endothelioid cell layer.

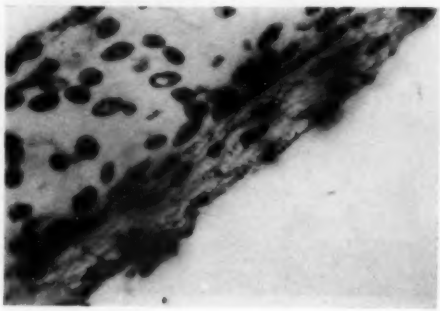


Photo. 11. Proliferation and thickening of endothelioid cell layer, and exudation of small round cells.

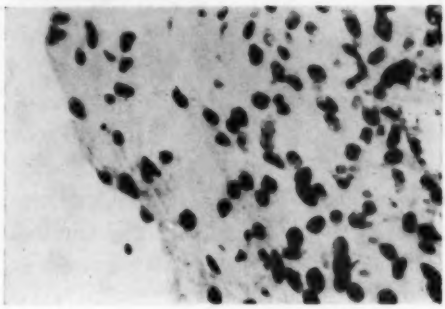


Photo. 12. Chromatolysis of endothelioid cells in its early phase.

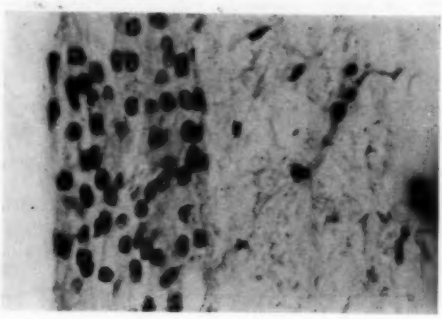


Photo. 13. Vascularization in endothelioid cell layer.

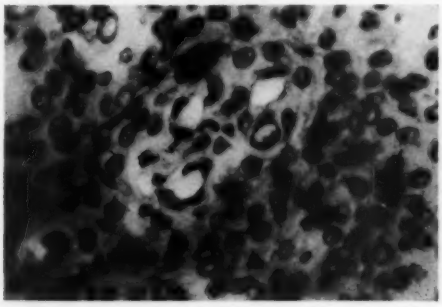


Photo. 14. Chromatolysis of endothelioid cells.

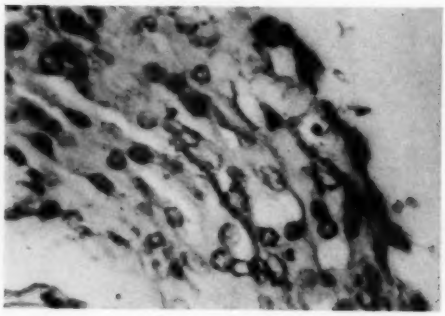


Photo. 15. Hyaline degeneration of the tunica media of small blood vessels in endothelioid.

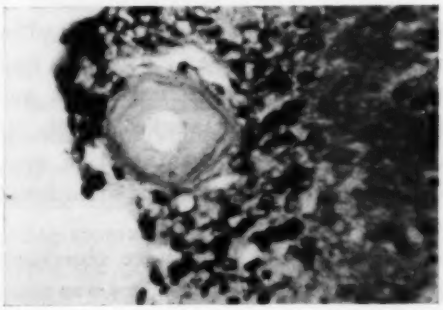


Photo. 16. Thickening of connective tissue layer.

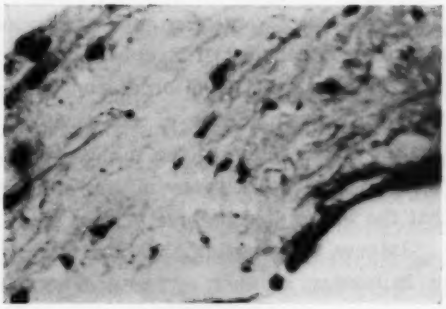


Photo. 17. Blood vessels in subarachnoid tissue and cavity (the arachnoid noted below).

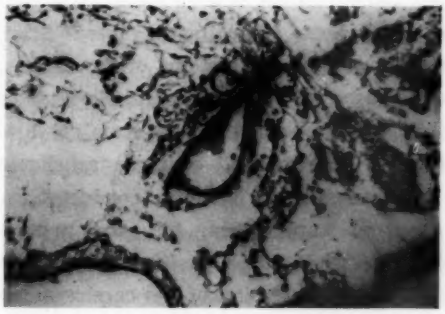
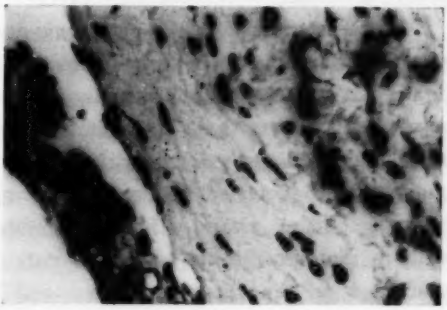


Photo. 18. Left, endothelioid cell layer; middle, connective tissue layer; upper right, subarachnoid tissue.



Thickening and proliferation of the connective tissue layer were rather mild in most cases, but in three cases these findings were either moderately or highly conspicuous. (Photo 7) Infiltration of lymphocytes was slight in many cases. (Photo 8) In many cases no emigration of lymphocytes was noted in the aubarachnoid tissue, but in a case of healed meningitis tuberculosa three months after onset of disorder lymphocytes had intensely emigrated, (Photo 9) and the tunica media of small blood vessels in the subarachnoid space showed a moderate thickening.

Six months to 1 year after onset of disorder (26 Caaes). Mild or moderate thickening and proliferation of the endothelioid cell layer were noted in almost all the cases. (Photo 10) Intercellular emigration of lymphocytes was noted in 4 cases. (Photo 11)

At such an interval after onset of disorder the number of cases who showed chromatolysis of endothelioid cells had increased, and this finding was demonstrated in almost all the cases to more or less degree. (Photo 12) In a case clinically diagnosed as arachnoiditis vascularization was clearly observed in the endothelioid layer. (Photo 13)

Thickening and proliferation of the connective tissue layer was noted in all the cases, and in some cases these findings were particularly marked. The number of

emigrating lymphocytes had rather diminished, compared with cases less than 6 months after onset of disorder.

More than 1 year after onset of disorder (*33 Cases*). Thickening and proliferation of the endothelioid cell layer were moderate in the majority of cases. At such a long interval after onset of disorder emigrating lymphocytes were hardly observable in any case, but chromatolysis of endothelioid cells had become rather marked. (Photo 14) Hyaline degeneration of the tunica media of small blood vessels was noted in some cases. Vessel walls were uniformly stained red with Van Gieson's stain, and so thickened that the lumen had become very narrow. (Photo 15)

Intense proliferation and thickening of the connective tissue layer were observable in increasing number of cases. (Photo 16) Mild infiltration of lymphocytes was noted in a very few cases. Thickening and proliferation of the subarachnoid tissue were either slight or moderate. In some cases emigrating lymphocytes were noted. (Photo 17) Several cases showed a moderate emigration of lymphocytes as well as very advanced proliferation and thickening. (Photo 18)

All these cases with the pathologic arachnoid had been clinically diagnosed either as retrobulbar neuritis in early phases of disorder, showing no symptoms in the fundus, or as optic neuritis or optic atrophy is some later phases. No roentgenographic findings were obtained from these cases, but they were diagnosed as arachnoiditis from cerebrospinal fluid findings and cerebral nervous sysmtoms. Indeed in all of them arachnoid changes were detected during the operation of craniotomy which was conducted for improvement of vision. In craniotomized cases of papilledema, too, the present authors macroscopically noted not only marked thickening and proliferation of the arachnoid on the cerebral surface and base, but also retention of the cerebrospinal fluid in the cisterna chiasmatica. Pathohistotological changes of the arachnoid were also clearly noted.

To sum up, fibrin deposits were noted in early phases of arachnoiditis. Emigrating cells consisted chiefly of lymphocytes, but in some cases emigration of polynuclears was also noted. Even 6 months or one years after onset of disorder emigration of these cells was noted in no small number of cases. The intensity of proliferation and thickening was generally proportionate to the length of interval after onset of disorder, but in some cases these findings were already conspicuous in relatively early phases. In later phases the nuclei of endothelioid cells fell into degeneration, and showed swelling and chromatolysis.

In some cases the subarachnoid tissue was examined, but the findings obtained were the same as those obtained from the arachnoid; namely, proliferation and thickening of trabeculae, emigration of cells, and fibrin deposits. In short, though various forms of cerebro-optic damage were pathohistologically investigated, no specificity was recognized in the arachnoid findings.

II. Circulation of the cerebrospinal fluid in various forms of cerebro-optic damage

(1) Secretion and absorption time of the cerebrospinal fluid in various forms of cerebrooptic damage

Methods. The examinee was made to lie quietly on the side for about 5-10 minutes, and his legs being sharply bent, a lumbar puncture was done. A glass tube of a glass manometer (marked off every 1 mm.) was held vertically. The examinee was then allowed to lie comfortably, the head being brought into a horizontal position to the long axis of the body, and the both legs being bent 45 degrees. Next, a 5cc. syringe was attached to one end of a stop-cock. The glass tube and the stop-cock were supported with a fixing apparatus. The primary pressure was recorded after 5-minute long observation when the pressure became stable at a certain level. Forty seconds or 1 minute later exactly 5 cc. of fluid was drawn. The final pressure was recorded 2 minutes after this drawal. Readings of pressure being done every 2 minutes, time needed for a return to the primary pressure was graphically recorded. This time was called secretion time. Next, 5 cc. of fluid drawn into the syringe was injected back into the spinal cavity at the same speed as was taken. Time needed for the postinjection pressure to return to the preinjection pressure was recorded, and called absorption time.

In some cases the fallen pressure did not return to the primary pressure within 30 minutes. When the pressure thus remained unchanged at a lower level for 5-10 minutes, recording of secretion time was discontinued. And with this lower level as the primary pressure, measurement of absorption time was commenced. Return to the primary pressure was achieved in all the normal cases, but not in many diseased cases, though recording was continued for a long time. With these cases, therefore, it was impossible to record secretion time, and time needed for return, too, varied with individual cases. Such being the case, the present authors had to calculate the ratio of the fallen pressure immediately after drawal of fluid to the pressure 15 minutes later, and called this ratio secretion rate. Absorption rate was likewise reckoned out. Continous variations in secretion and absorption curves with the passage of time were also investigated. Taking all the above-mentioned data together into consideration, all the cases were classified into 4 type; a,b,c, and d. By "normal" in the notes of Table I are meant those cases who needed on an average 12-18 minutes for return to the primary pressure, and whose absorption time was 11-19 minutes; by "shortened" those who required less time than the above-mentioned; and by "prolonged" those who required more time.

With normal cases the mean time and standard deviation of secretion was 15.60 ± 3.25 minutes, while those of absorption 15.60 ± 3.25. A balance was thus noted between secretion and absorption in normal cases. On the contaray, with regard to diseased cases, this balance was lost, and secretion and absorption were either shortened or prolonged. In cases of retrobulbar neuritis, Leber's disease, and optic atrophy secretion time was commonly shortened in early period after onset of disorder, but chronic cases commonly showed prolonged secretion and absorption. Particularly secretion

Table I. Classification of Cases based on Collective Judgment of Secretion and Absorption Time

	SECRETION TIME	ABSORPTION TIME	RETROBULBAR NEURITIS	LEBER'S DISEASE	OPTIC ATROPHY
a	↓	↓	15	8	14
b	↑	n~↓	9	4	3
c	n	↓	9	3	2
d	↓	n~↓	8	1	6

Notes: ↑ secretion (absorption) time shortened; ↓ " " prolonged; n " " normal

time showed a tendency to marked prolongation. Prolongation of both secretion and absorption became more conspicuous with the passage of time.

(2) Uranin permeability test in cerebro-optic damage

Methods. A 10% solution of uranin (fluorescein natrium) was injected into the right v. mediana 0.2 cc/kg. quantities. Exactly 30 minutes later a lumbar puncture was done, and 8.0 cc. of fluid was accurately drawn. The drawn fluid was poured into a 8.0 cc. cubet, and measured for uranin content with a fluorophotometer which had been previously adjusted to indicate 100 to a 0.00001% standard solution of uranin. The cerebrospinal fluid itself was measured for blank fluorescence, and its value of blank fluorescence was deducted from the experimentally obtained value of uranin concentration.

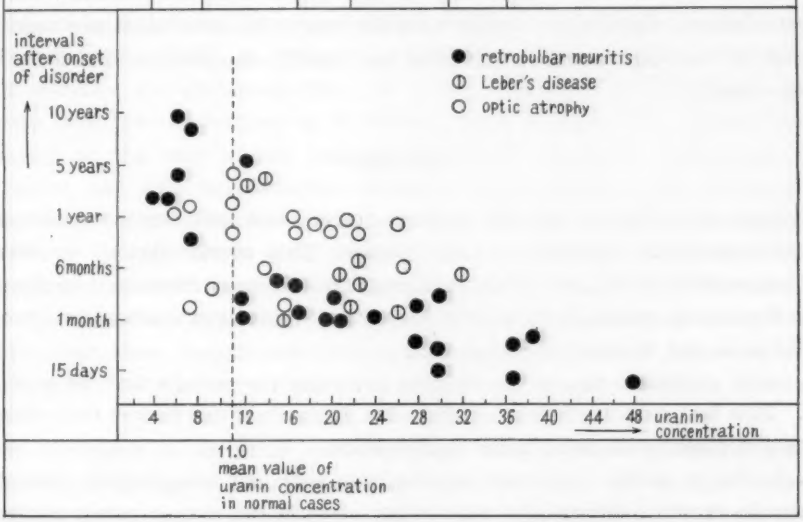
Results. The mean value and standard deviation of uranin content of the fluid was 11.02±3.14 in 35 normal cases. Cases of retrobulbar neuritis, Leber's disease, and optic atrophy (simple or postinflammatory of unknown etiology) were divided into three groups according to length of interval (less than 1 month, 1 month-1 year, and more than 1 year) after onset of disorder, and fluorophotometrically examined. Results are given in Table II.

As shown in the figure appended to Table II, uranin permeability was markedly increased in early phases of these diseases, but it either approached the normal value, or rather tended to decrease below normal with the passage of time. Twelve cases of fundus diseases which had nothing to with retrobulbar neuritis were also subjected to this test as controls, but in these cases uranin permeabillity did not change through the whole long course of illness. Thirty craniotomized cases, too, underwent this test, and it was found that permeability had markedly increased in all the cases in comparison with preoperative values. Even one month after operation permeability was maintained at higher levels.

Animal experiments and results. Adult rabbits weighing 2.0-2.5 kg. were used. A 10% solution of uranin was injected into the right ear vein in 0.2 cc./kg. quantities. After a certain period of time less than 1.5 cc. of the cerebrospinal fluid was drawn

Table II. Mean Values and Standard Deviations of Uranin Test at Various Intervals after Onset of Optic Disorder, and Distribution Dots

TYPES OF OPTIC DISORDER	CASES	LESS THAN 1 MONTH	CASES	1 MONTH-1 YEAR	CASES	MORE THAN 1 YEAR
RETROBULBAR NEURITIS	7	35.02±6.48	12	19.27±5.59	7	8.42±1.72
LEBER'S DISEASE	0		7	23.57±6.05	2	12.16
OPTIC ATROPHY	0		13	18.00±5.77	6	15.62±5.97



by puncture of the cisterna magna. We had to be content with this amount, as the fluid was often soiled by bleeding. After measurement of amount, the drawn fluid was diluted with aqua destillata to 8.0 cc., and poured into a cubet. Later procedures were the same as those used in clinical experiments.

Uranin content reached the maximum 26-28 minutes after injection in 35 normal rabbits. In these animals the mean value and standard deviation of uranin permeability was fluorophotometrically 76.2±16.2.

In cases of experimental talcum-powder arachnoiditis (which had been produced by injecting a well-mixed suspension of 250 mg. talcum powder in 1.0 cc. of isotonic saline solution in 0.2 cc./kg. quantities after drawal of 1.0cc. of the fluid from the cisterna magna) markedly increased permeability was noted 1 and 3 months after injection. Even 6 months later permeability was still very high.

Specimens were further prepared from the chiasmal arachnoid, and examined. No. marked changes were demonstrated in the arachnoid over the fornix cerebri six months after production of talcum-powder arachnoiditis, but the membrane over the basis

cerebri was extensively involved, and the endothelioid cell layer around the optic chiasm in particular showed moderate thickening and proliferation. Nuclear swelling and chromatolysis were also noted. Numerous needlelike crystals of talcum power were sticking in the markedly proliferated connective tissue layer, and lymphocytes, plasma cells and polynuclears, too, were present in small numbers. This was clearly a picture of foreign-body inflammation accompanied with degenerative changes. Hydrocephalus internus was recognized in the specimens of cerebrum, and this seems to have developed owing to the presence of circulation disturbance of the cerebrospinal fluid. Epithelial cells of the ventricular vascular plexus were swollen, and their nuclei had lost uniform stainability. Many vacuoles were also noted in theses degenerating cells. As for the optic nerve, it had fallen into Marchi degeneration, and demyelination was also noted.

Summary

In cases of retrobulbar neuritis, Leber's disease, and optic atrophy uranin permeability was remarkably increased in early phases. This acceleration of secretion, and rise of permeability on the part of the cerebrospinal fluid is attributable to inflammation. And as the rise of uranin permeability is observable also after craniotomy, it may be regarded as a part of curative mechanism.

In such a fundus disease as retinitis centralis the uranin test gives a normal result. This fact may be utilized in differential diagnosis of this disease from retrobulbar neuritis and others with intracranial arachnoiditis,

It should be stated here that arachnoiditis does not result solely from curative processes in cerebral diseases and retrobulbar neuritis, for even in purely mechanically-produced talcum-powder arachnoiditis markedly increased uranin permeability was already noted in early phases, and moreover, a secondary development of degeneration and demyelination was demonstrated.

Discussion

In many cases of various cerebro-optic damage investigated by the present authors, marked fibrin deposits, and emigration of lymphocytes, plasma cells and sometimes polynuclears were noted in the arachnoid tissue in early period after onset of disorder. Similar findings were also obtained from the subarachnoid tissue. With these findings the present authors demonstrated the existence of exudative changes in the arachnoid in various forms of cerebro-optic damage. On the other hand, increased permeability of the cerebrospinal fluid could be inferred from increase of uranin content.

Concerning the etiology of arachnoiditis it is considered either that a noxe of some sort may hematogenously act upon the vascular plexus, and increase permeability of the fluid, or that a noxe discharged into the fluid may secondarily cause arachnoiditis.

Factors responsible for increase of uranin permeability may be many, but pathologic changes were noted in the arterioles and capillaries of the arachnoid. It is further considered that thickening and proliferation of the arachnoid resulting from chiasmal arachnoiditis may bring about circulation disturbance of the fluid; and that this circulation disturbance may on its part disturb metabolism of the optic nerve, and thus impair the function of the latter together with a noxe which exerts a direct injurious action.

In normal cases invetigated by the present authors the mean value of secretion time was nearly the same as that of absorption time. This fact indicates that a stable balance is maintained between secretion and absorption in the normal circulation of the cerebrospinal fluid. In cases of various cerebro-optic damage this balance is lost, and secretion and absorption time are either shortened or prolonged. Many of these cases have been ascertained to be suffering from arachnoiditis. Besides increased permeability on the part of the inflamed arachnoid, absorption disturbance in the spinal cavity was also demonstrated. Quantitatively-measured uranin content of the fluid, and secretion and absorption time permit us te make a fair estimate of time after onset of disorder, for in early phases of disorder secretion is increased, but with the passage of time both secretion and absorption, particularly the latter, come to be more disturbed and more prolonged. Long years after onset of disorder secretion and absorption time show conspicuous prolongation. As for uranin permeability, it is considerably increased in early phases, but half a year or one year later it is restored to normal.

When variatiors in secretion and absorption time, results of uranin test, and pathohistological findings of the arachnoid are together taken into account, genesis of arachnoiditis, and essential nature of thereby-caused optic damage become systematically understandable. Sometimes, however, uranin permeability remains unnormalized for a considerably long time, and in the meantime arachnoid damage becomes intensified with the progress of chronic inflammation. Under such circumstances the circulation and absorption of the fluid is naturally disturbed with a result that uranin discharged into the fluid continues to stay there. In such a case the problem of uranin concentration becomes very complicated.

In all the cases of arachnoiditis craniotomized for the sake of improving vision uranin permerbility was greatly increased in comparison with preoperative values. This fact shows that freeing and exposure of the optic chiasm improve the circulation of the cerebrospinal fluid. This betterment of the circulation will necessarily promote the tissue metabolism of the optic nerve, thereby contributing to effecting a cure.

The present authors further noted in rabbits with experimental talcum-powder arachnoiditis that uranin concentration was markedly increased in the fluid of the cisterna magna. In the specimens prepared from the chiasmal arachnoid, vascular plexus, and ventricular wall of these rabbits, intense and advanced arachnoid infla-

mmation due to foreign-body irritation was noted. Connective tissue cells and trabecular tissuues had developed such severe proliferation and adhesion that the circulation of the fluid was greatly obstructed. With the passage of time endothelioid cells of the arachnoid showed chromatolysis, and finally fell into complete degeneration. Uranin permeability, too, was gradually reduced from the first month to the 6 th month after production of arachnoiditis. These results of pathohistological investigations as well as uranin test are quite the same as those in human cases of arachnoiditis. Moreover, as Imachi had already stated in his special lecture in a general meeting of the Japanese Ophthalmological Society in 1957, 6 months after production of arachnoiditis advanced Marchi degenertion and demyelination of the optic nerve and the spine were demonstrated in these expeimental cases, just as in human cases of retrobulbar neuritis and other demyelinating diseases.

Conclusions

In about 300 human cases of retrobulbar neuritis the arachnoid was pathologically investigated, and circulation disturbance of the cerebrospinal fluid was demonstrated. The pathological findings obtained from animal cases of experimental arachnoiditis were quite the same as those obtained from human cases. It was experimentally confirmed that arachnoid damage very often witnessed in retrobulbar neuritis is not always a secondary development, but can be a causative factor of retrobulbar neuritis.

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ELECTRON MICROSCOPIC OBSERVATION OF THE OPTIC NERVE

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In 1957 Jo Imachi delivered a special lecture on neuritis retrobulbaris at a general meeting of the Japanese Ophthalmological Society. Since then our laboratory has continued experimental pathohistological investigations on this disease under the direction of Prof. Imachi. Owing to the limited resolving power of the optical microscope, however, fundamental anatomical problems such as the structures and properties of

the axon cylinder and the myelin sheath, demyelinating process, the morphology and degeneration of glia cells, and the relation of blood vessels to perivascular tissue have always defied our attempts at complete solution. As a first step to morphological clarification of the true nature of neuritis retrobulbaris, the present author has investigated the ultrastructure of optic nerves of normal mice and rabbits with use of an electron microscope. Results obtained are given below.

Materials and Methods

The materials were taken from the optic nerves of adult mice and rabbits. The tissues were fixed in 1%-buffered osmium teroxide solution. After fixation, they were dehydrated through a graded series of ethanol and embedded in n-butyl-methacrylate : methylmethacrylate (7 : 3) at 45°C. The electron microscope used was Tronscope 50 of AKASHI Company.

Results

The normal optic nerve is composed of nerve fibers, septal connective tissues, glia cells and blood vessels.

I. Nerve Fiber

Components of nerve fiber (*Fig. 1, 2, 3, and 11*). The nerve fiber is composed of the axon cylinder and the myelin sheath encircling the former. In the axon cylinder axon filaments, endoplasmic reticulum, and mitochondria are always observable, and these are surrounded by the axon membrane. Axon filaments are generally about 100 Å in diameter, and look dotlike in a cross section of the optic nerve, while in a longitudinal section they are seen running straight, in places obliquely, along the long axis of the optic nerve. They anastomose with one another to form a meshwork.

In the axon cylinder, on the other hand, was not noted a fibrous structure corresponding in thickness to the so-called neurofibril which is recorded as optical microscopically observable.

Endoplasmic reticulum are 500-2000 Å in size.

As to mitochondria, they measure 0.1-0.5 μ, and being thus not uniform in size, they assume various circular shapes in a cross section, while in a longitudinal section linear forms.

Around axon cylinders there are axon membranes which are 100-150 Å in size. These membranes pass over into myelin sheath lamellae. In the myelinated nerve fiber the myelin sheath encircles the axon cylinder, and in a cross section shows a high electron density, and look like a piece of ribbon. Under greater magnification its ultrastructure is found to be consisting of lamellae with high electron density and those with low electron density. These two different lamellae are piled upon each other in alternating layers. Low density lamellae are larger in width, and their periodicity is about 140 Å. (Fig 2,5)

Fig. 1. A cross section through the optic nerve of mouse. The myelin sheath (Ms) encircles the axon cylinder (Ax). In the axon cylinder axon filaments (Axf), endoplasmic reticulum (e) and mitochondria (m) are observable, and these are surrounded by the axon membrane (Axm). (Gp): glial processes. ×72,000

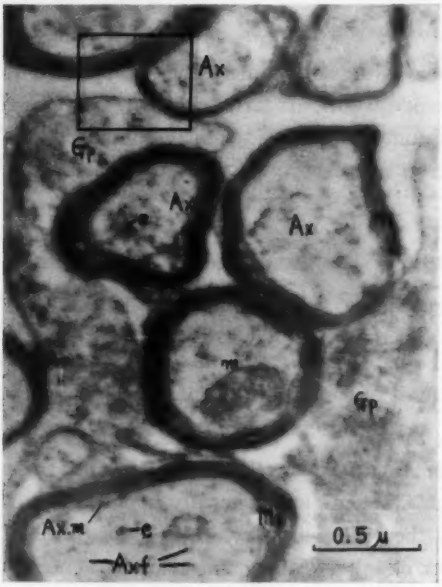
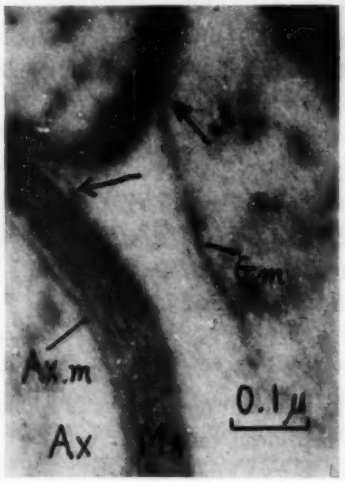


Fig. 2. A myelin sheath in Fig 1 at higher magnification. Not the alternating dark and light lamellar structure of myelin sheath. ×142,000



Thickness of nerve fiber. In both rabbits and mice myelin-sheathed nerve fibers are 0.1–3.0 μ in diameter, and the majority of them (81% with rabbits, and 96% with mice) less than 1 μ. Myelinated sheaths are 0.04–0.28 μ in thickness with rabbits, and with mice 0.03–0.25 μ, the majority of them being less than 0.1 μ. Their thickness is generally proportionate to that of nerve fibers.

Properties of nerve fiber (Fig. 4,6). Nerve fibers in the optic nerve are mostly myelinated, but in the peripheral part of the nerve one non-myelinated nerve fiber about 0.3 μ in length was noted for about 40 myelinated ones. This non-myelinated fiber possesses only the axon membrane, and is enveloped along its entire circumference by glial processes.

In the myelinated fiber the outer layer of the axon membrane turns outward after encircling the inner layer. These two layers attaching to each other envelope the axon cylinder in the form of a spiral. The membrane then goes over into myelin sheath lamellae. And the outermost lamellae, parting from nerve fibers, either come into contact with the same of other myelin sheaths, or pass into glia cell membranes, thus forming a sort of membranous system. By the way, the so-called node of Ranvier could not be located in the present investigation.

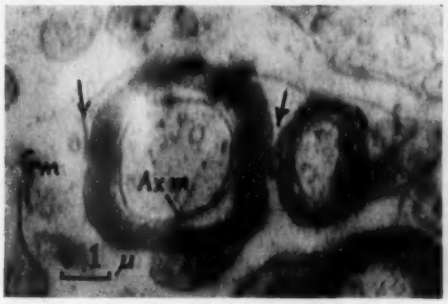
Ⅱ. Glia Cells

In the present investigation glia cells in the optic nerve were classified into three

Fig. 3. A longitudinal section of axon cylinder. ×18.200

Fig. 4. A cross section through the optic nerve of mouse. A non-myelinated nerve fiber (n-Ms) are observable. ×36,000

Fig. 5. A myelin sheath in Fig 4 at higher magnification. ×114,000



types from a morphological point of view.

Glia cells of the first type (*Fig. 7*). Glia cells of this type are most numerous. The nuclei are either nearly circular, or elliptical, and 3-4 μ in diameter with mice, and with rabbits 6-8 μ. Nucleoli are also rarely observable. The nucleoplasm is thick, and the nucleus and the cytoplasm show a moderate electron density. The cytoplasm is small in size, compared with the nucleus. The cell membrane is in direct contact with the adjacent nerve fibers, and contains mitochondria, endoplasmic reticulum, and Golgi's apparatuses. The cell is in possession of many processes which run to a relatively far distance. These processes also have mitochondria.

Glia Cells of the Second Type (*Fig. 8*). The nuclei are mostly elliptical, and about 2×3 μ in size with mice, while with rabbits about 6×7 μ. The nucleoplasm is relatively rough, and the electron density of the nucleus and the cytoplasm is rather lower than

Fig. 6. A cross section through the optic nerve of mouse. A non-myelinated nerve fiber (n-Ms) possesses the axon membrane, and is enveloped along its entire circumference by glial processes (Gp). ×34,000

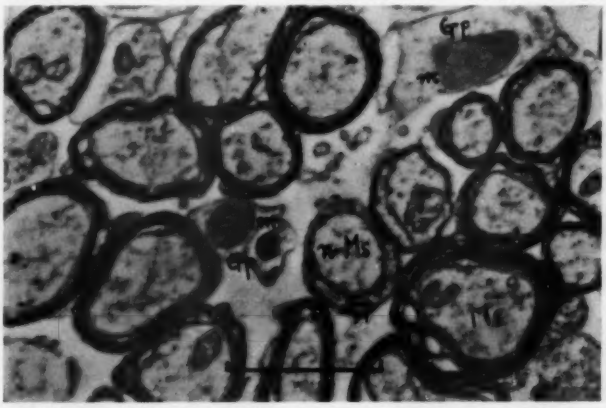


Fig. 7. A glia cell of the 1st type. N: nucleus. ×17,400

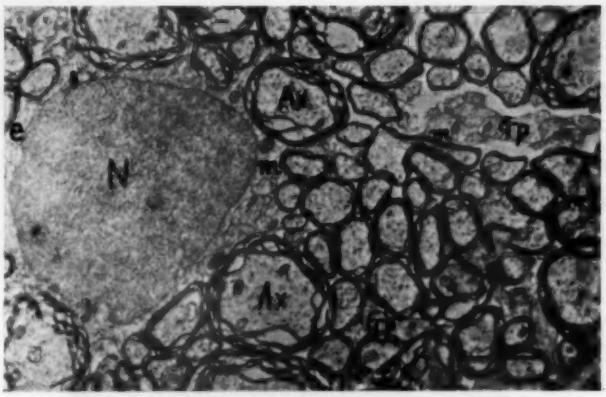


Fig. 8. A glia cell of the 2nd type. ×9,800

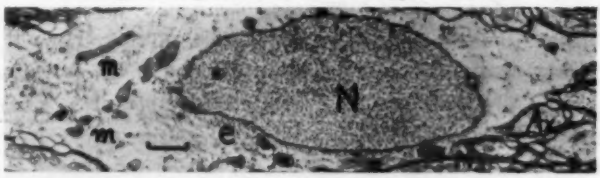


Fig. 9. A glia cell of the 3rd type. ×15,000

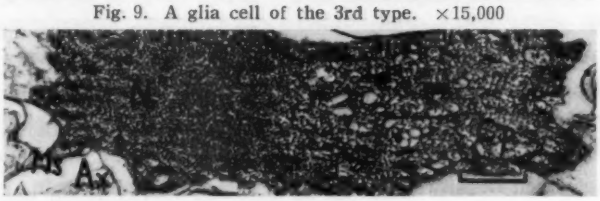


Fig. 10. A glia cell of intermediate type between the 1st and 2nd type. ×12,800

Fig. 11. A longitudinal section through the optic nerve of mouse. ×42,000

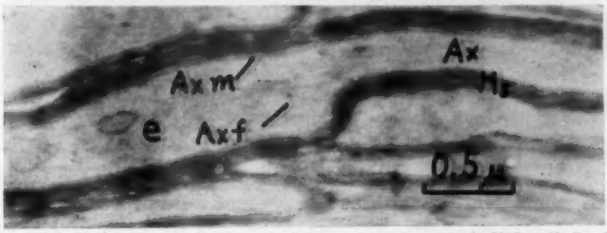


Fig. 12. A capillary in the optic nerve of mouse. Et: endothelial cell. L: lumen. Bm: basement membrane. R: red blood cell. P: perivascular cell. ×17,600

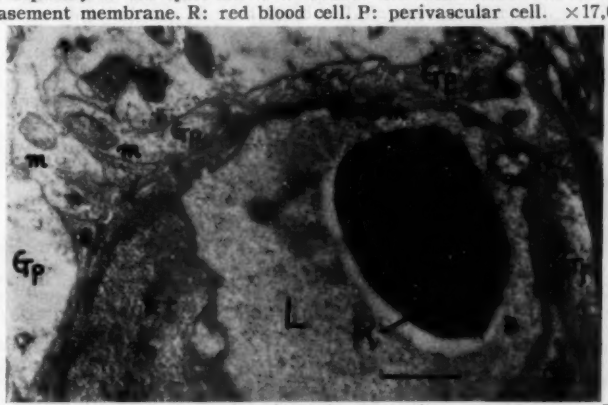


Fig. 13. The septal connective tissue in the optic nerve of mouse. Ct: connective tissue. ×19,600

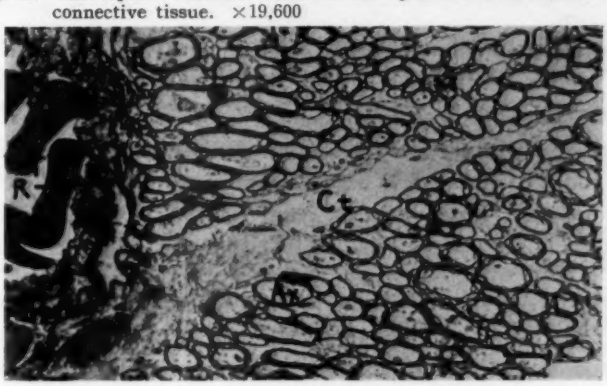
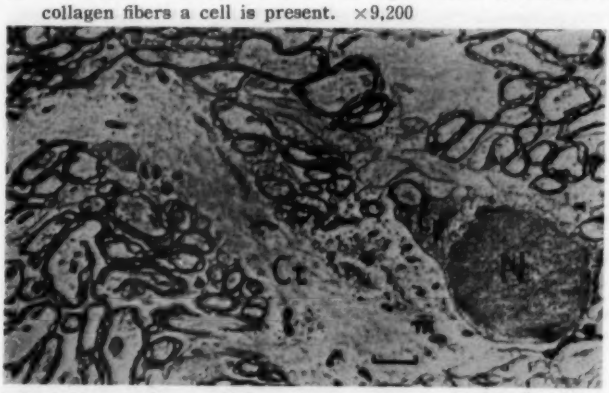


Fig. 14. The septal connective tissue in the optic nerve of mouse. Among collagen fibers a cell is present. ×9,200



in the case of first-type cells. This cell is rich in watery cytoplasm, and in it mitochondria and endoplasmic reticulum are present. Its processes are small in number.

Glia Cells of the third type (*Fig. 9*). The cell and its nucleus are both irregular in shape, and have indistinct boundaries. Their electron density is very high. Cell processes are short, and hemmed in between nerve fibers, and seem to pass into the outer wall of the myelin sheath. The internal structure of the cell is not clearly observable, but the presence of endoplasmic reticulum was noted.

Besides the above mentioned glia cells, those of intermediate type between first and second types were also noted. (Fig. 10)

Ⅲ. Blood Vessels (Fig. 12)

A few blood vessels are observable in the septal connective tissue, and in the space between nerve fiber bundlef. In the capillary between nerve fiber bundles endothelial cells are encircled by the basal membrane in which perivascular cells are present. This membrane is again encircled by glia cells apparently of the first type along its almost entire circumference. The processes of these glia cells possess mitochondria. Endothelial cells are closely connected with the basal membrane, leaving no space between them.

Ⅳ. Septal Connective Tissue (Fig. 13, 14)

The septal connective tissue runs from the the peripheral pial membrane into the optic nerve chiefly along the capillary-containing peripheral part of the optic nerve. In some places it is easily distinguished from the adjoining nerve fiber bundles, but not so in other places. Many mitochondria are observable in it which is filled with collagen fibers. Among collagen fibers cells of relatively low electron density are present. These cells are poor in cytoplasm, and their nuclei are either elliptical or irregular in shape.

Discussion

In electron microscopic examinalion the fixation of nerve tissue is regarded as much

more difficult than that of other tissues. Particularly with regard to the myelinated nerve fiber, the preservation of its myelin sheath has taxed the ingenuity of many investigators, and various fixative solutions have been tried. Of these fixative solutions, a buffered osmium solution is now regarded as the best. In the beginning stage of investigation the present author, too, tried many fixative solutions, and made studies on pH and osmotic pressure, but he could not ascertain during what process in fixation and polymerization, and through what mechanism artificial disintegration of the myelin sheath so frequently occurred.

Author once conducted polymerization experiments, using polyester and methacrylate under the same fixation conditions, and happened to note that artificial disintegration of the myelin sheath occurred with the latter, but not with the former. After many experiments of this kind author found that preparation of materials, fixation time, types of buffer solutions, and pH had almost no bearing on disintegration; that is, that disintegration of the myelin sheath depended solely on the relationship between contraction rate and polymerization heat during the process of polymerization. Results of these polymerization experiments with polyester will be published later in another paper.

The significance of electron microscopy of the optic nerve lies in that we can observe the axon cylinder, the myelin sheath, and glia cells simultaneously under a condition rather similar to a living condition. Formerly we had to use various staining methods, and observe them separately.

As the presence of axon filaments, mitochondria, and endoplasmic reticulum in the axon cylinder may be taken as an indication of commencing changes, and as myelin sheath lamellae are now clearly observable, detection of fine changes during an early stage of demyelinating process has become quite possible.

It should be especially emphasized that findings corresponding to the so-called neurofibrils, the existence of which has till now been regarded as an established fact, could not obtained by the present author. Honjin stated from results obtained from his experimental cases that long fibrous structures were formed along the long axis of the nerve during fixation in alcohol and formalin in the case of optical microscopic examination; and that endoplasmic reticulum, mitochondria, and axon filaments, being condensed during the process of fixation and dehydration, participated in the formation of these structures. He further asserted that these dehydrated materials, if stained with silver, absorbed silver particles, and were mistaken for neurofibrils. This, Honjin's report teaches us that electron microscopy is of vital importance for correct estimation of fibrous changes in the optic nerve.

Inner myelin sheath lamellae are connected with the axon membrane, and encircle the axon cylinder, while the outermost ones leave the myelin sheath, and pass over into the membranes of glial cells. From this fact it is considered that not the axon cylinder, but glia cells take part in the growth of myelin sheaths.

The thickness of nerve fibers was formerly estimated through optical microscopy

to be between 0.5 and 10 μ, but these values have a wide range of error, and can not be trusted. The present author has ascertained that the majority of nerve fibers in rabbits and mice are less than 1 μ in diameter. This finding may be regarded as a morphological corroboration of impulse conduction in physiology.

The doubt about the existence of non-myelinated nerve fibers in the optic nerve was also cleared up by the present observation. That is, the non-myelinated nerve fiber possesses only the axon membrane, and its axon cylinder has an internal structure similar to that of the neighboring myelinated nerve fibers. It can be differentiated from glial processes in that it has a different internal structure, and is bounded by double structure surrounded by glial processes.

As to types of glia cells, the present author ignored the traditional classification into three kinds, as he did not know how these cells would appear under an electron microscope, and had to place them under the first, second, and third types. Adequate classification of these cells is left for future researches.

Blood vessels are encircled by glial processes along their entire circumference, and no space is findable between endothelial cells and the basal membrane. Concerning the septal connective tissue, many details still remain unexplored. Inquiry into them is a problem for future investigation.

Summary

The optic nerves of normal adult mice and rabbits were investigated with electoron microscope.

1. In the axon cylinder axon filaments, endplasmic reticulum, and mitochondria are always obervable. Axon filaments are generally about 100 Å in diameter.

2. The periodicity of myelin sheath lamella is about 140 Å.

3. In both rabbits and mice myelinated nerve fibers are 0.1 μ-3.0 μ in diameter, and the majority of them (81% with rabbits, and 96% with mice) less than 1.0 μ.

4. In the peripheral part of the optic nerve non-myelinated nerve fiber about 0.3 μ in length was noted.

5. Glia cells were classified into three types.

a. The 1st type

The nuclei are either nearly circular or elliptical. The nucleoplasm is thick, and the nucleus and the cytoplasm shows a moderate electron density. The cytoplasm is small in size, compared with the nucleus. The cell is in possession of many processes which run to a relatively far distance.

b. The 2nd type

The nuclei are mostly elliptical. The nucleoplasm is relatively rough, and the electron density of the nucleus and the cytoplasm is rather lower than in the case of the 1st type cells. This cell is rich in watery cytoplasm. Its processes are small in number.

c. The 3rd type

The cell and its nucleus are both irregular in shape, and have indistinct boundaries. Their electron density is very high. Cell processes are short.

6. In the capillary between nerve fiber bundles endothelial cells are encircled by the basal membrane in which perivascular cells are present. This membrane is again encircled by glia cells apparently of the first type along its almost entire circumference.

7. The septal connective tissue is easily distinguished from the adjoining nerve fiber bundles. Many mitochondria are observable in it which is filled with collagen fibers. Among collaged fibers cells of relatively low electron density are present.

As seen in the above, the ultrastructure of the optic nerve, which is unobservable with an optical microscope, has been gradually unveiled by electron microscopic observation. Keeping abreast with technical improvements in electron microscopy, the present author intends to carry out investigation into glia cells and septal connective tissue, both of which still remain practically unascertained. In co-operation with M. Murai and T. Komai, the present author is now making electron microscopic studies of Wallerian degenration, and neuritis retrobulbaris caused by various forms of toxicosis and vitamin deficiency. Results of these studies will be published shortly.

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ELECTROMYOGRAPHIC STUDY ON RETRACTION SYNDROME

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The cardinal symptoms of retraction syndrome are complete abolition or restriction of abduction and marked retraction of the eye on attempted adduction.

It is generally known, that the lateral rectus is often an inelastic and fibrous band and this may limit adduction and cause retraction. Some authors believe that there are abnormalities of the fifth nerve nucleus, but it has not been proved yet.

Breinin reported that abnormal action potentials were obtained on adduction from the lateral rectus of the retraction syndrome, and he thought that it was due to a stretch reflex.

We examined 14 cases of retraction syndrome electromyographically. The purpose of this paper is to report the results.

Method

The chief aim of this examination was to know how action potentials of the extraocular muscles of retraction syndrome changed on various eye movements. The method was the same as Mommose described before; that is, bipolar needle electrodes, under local anesthesia, were inserted through the conjunctiva into the muscles. Action potentials induced from the muscles were led to an electromyograph with push-pull type amplifier; Further, one of the outputs was directly, and the other was through the integrator, led to an oscillograph of the electromagnetic type; the direct EMG wave and integrated EMG wave were simultaneously recorded on a moving oscillographpaper.

In addition to bipolar needle electrodes, in order to obtain action potentials of a single motor unit, a concentric needle electrode was used.

Table 1. The Fluctuations of Action Potentials of the Extraocular Muscles of Retraction Syndrome on Eye Movement

			muscle	medial rectus		lateral rectus				superior rectus		inferior rectus	
			movement	addu-ction	abdu-ction	addu-ction	abdu-ction	supra-duction	infradu-ction	addu-ction	abdu-ction	addu-ction	abdu-ction
Case No.	age	sex	side										
1	21	♂	left	⫴	−	++	+	+	++				
2	55	♀	left			⫴	−	++	++	++	++	++	+
3	16	♂	right	++	−	+	++	++	+			++	+
4	20	♂	left	++	−	++	+						
5	22	♂	left			⫴	⫴					++	+
6	17	♀	left			⫴	⫴			++	+		
7	33	♀	right	⫴	−	⫴	+						
8	21	♂	left			−	−			++	+		
9	20	♀	right			−	−						
10	19	♀	right			+	++						
11	22	♀	left			⫴	++						
12	18	♀	left			⫴	⫴						
13	16	♂	left			⫴	⫴						
14	20	♀	left			⫴	++						

The marks (⫴, ++, +, and−) show the relative increase or decrease of action potentials of each muscle on horizontal or vertical movement. Action potentials of extraocular muscles of a patient should not be compared in the table with that of the other patient.

Results and Discussion

14 cases of retraction syndrome were examined electromyographically. The lateral rectus was examied in all cases, but the other extraocular muscles only in a few cases (Table 1).

Action potentials were obtained from the lateral rectus in 12 cases, but in two cases were not obtained. In these two cases it seems that the lateral rectus was completely fibrous or electrodes did not touch the muscle fibers, though they existed.

In 6 cases out of 12 cases in which action potentials were obtained from the lateral rectus, action potentials of the lateral rectus increased as the eye adducted (Fig. 1-A, 3, 12-A).

In 4 cases out of 12, action potentials did not change in total quantity on horizontal movement, that is, action potentials on abduction were equal to action potentials on adduction (Fig. 9, 13-A).

In two out of 12 cases, action potential of the lateral rectus decreased as the eye adducted, but they were much more than that of the normal eye (Fig. 7-A).

It is obvious that abnormally many action potentials are obtained on adduction from the lateral rectus of retraction syndrome.

Breinin described that this phenomenon was due to a stretch reflex. To confirm his opinon, the following experiments were done in 4 cases (Cass 11, 12, 13 and 14).

The examinee was ordered to gaze at a target in front of him with the unexamined eye in order to prevent the fluctuation of action potentials of extraocular muscles due to any impulses from the supranuclear region. Then, the examined eye was passively adducted by fixation forceps. But in spite of the stretch of the lateral rectus there was no change of action potentials of the lateral rectus in every case (Fig. 12-c, 13-c). Then the examined eye was steadied by fixation forceps so that it was unable to move and the other unexamined eye was ordered to abduct so that the examied eye received the impulse of adduction. The action potentials of the lateral rectus of the examined eye increased slightly in case 11 and 14 (Fig. 12-B), and did not change at all in case 12 and 13 (Fig. 13-B). When the examined eye was not steadied, on voluntary adduction, action potentials of the lateral rectus increased in case 11 and 14 (Fig. 12-A), and did not show the fluctuation in case 12 and 13 (Fig. 13-A).

If the abnormal action potentials of the lateral rectus on adduction are due to stretch reflex, they must disappear when the examined eye is steadied and the other unexamined eye is ordered to abduct, since the lateral rectus is not stretched and the impulse from the supranuclear region, which the lateral rectus receives, should diminish. However, action potentials of the lateral rectus increased in two cases and did not change in the other two cases. Moreover, abnormal spike discharges, that is, low amplitude NMU voltage, fibrillation voltage, fasciculation voltage, complex NMU voltage, synchronization voltage, reinnervation voltage, and grouping voltage were not detected.

It is obvious that abnormal action potentials of the lateral rectus of retraction

syndrome are not due to stretch reflex, but to the abnormalities of the fifth nerve nucleus or the supranuclear region.

The quantitative changes of action potentials of the lateral rectus were investigated not only on horizontal movement of the examined eye, but also on vertical movement and on oblique movement with case 1 and 3.

Case 1: Action potentials of the lateral rectus increased as the eye adducted on the horizontal movement, and as the eye rotated from out-and-up to in-and-down and from in-and-up to out-and-down on oblique movement, and as the eye rotated down on vertical movement (Fig. 1-A,B,C,D).

Case 3: Action potentials of the lateral rectus increased slightly as the eye abducted on the horizontal movement, and as the eye rotated from in-and-down to out-and-up and from out-and-down to in-and-up on oblique movement, and as the eye rotated up on vertical movement (Fig. 7-A,B,C,D).

Ordinarily in the normal eye, action potentials of the lateral rectus are much more when the eye rotates out than when the eye rotates inwards, and more when the eye rotates out-and-up than when the eye rotates in-and-down, and more when the eye rotates out-and-down than when the eye rotates in-and-up. But our examinations showed that this fact does not apply to retraction syndrome and there are abnormalitis of the fifth nerve nucleus of the supranuclear region. Action potentials of the medial rectus of retraction syndrome increased on adduction and decreased on abduction normally (Fig. 2).

In order to study whether the view that the superior rectus and the inferior rectus take part in retraction on adduction is proper or not, the fluctuation of action potentials of these muscles on horizontal movement were investigated.

The superior rectus was investigated in only three cases, and action potentials of the superior rectus increased on adduction in two cases (Fig. 4,11).

The inferior rectus, also, was investigated in only three cases, and action potentials increased on adduction in all cases (Fig. 5,6).

Consequently the superior rectus and the inferior rectus of retraction syndrome seem to contract more and more strongly as the eye adducts. But it is difficult to conclude in this way, since the cases are very few.

It is an interesting problem whether the increases of action potentials, or the increases of contraction, of the superior rectus and of the inferior rectus and of the lateral rectus on adduction take part in retraction of the eye.

From the fact that retraction occurs even when the lateral rectus are completely fibrous or the action potentials of the lateral rectus can not be obtained, it is evident that retraction occurs in these cases without contraction of the lateral rectus.

It is sometimes seen in the retraction syndrome that, in spite of marked action potentials of the lateral rectus on adduction, only very slight retraction occurs in some of these cases and retraction of the eye often occurs on attempted adduction after

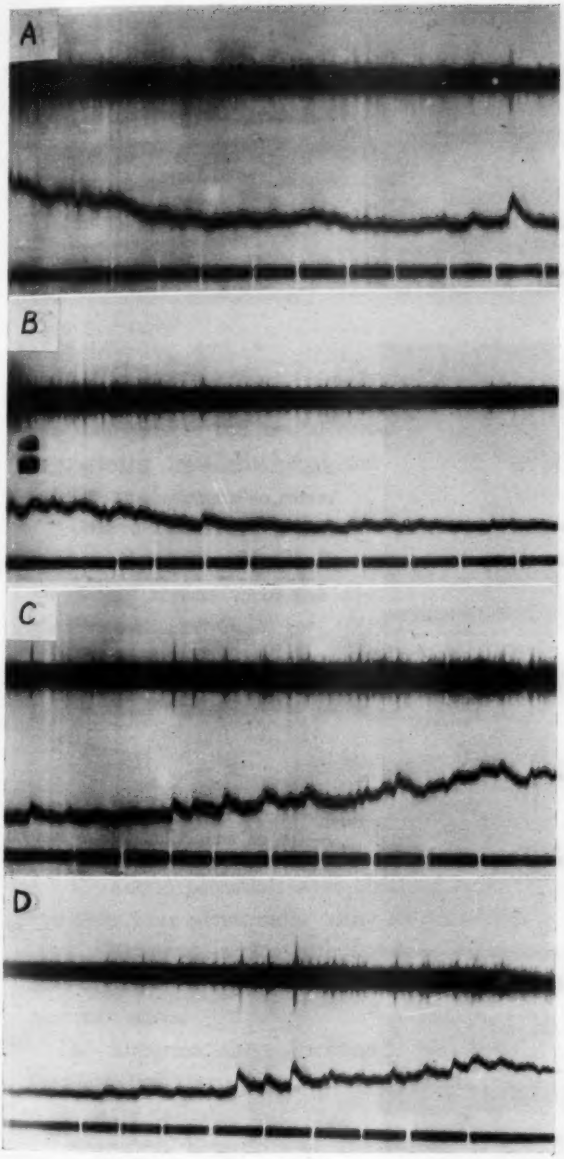

Fig. 1. (Case 1.) EMG of laterl rectus on various eye movement.
A. Action potentials decrease as the eye abducts.

B. Action potentials decrease as the eye rotates from out-and-down to in-and-up.

C. Action potentials increase as the eye rotates down.

D. Action potentials increase as the eye rotates from out-and-up to in-and down.

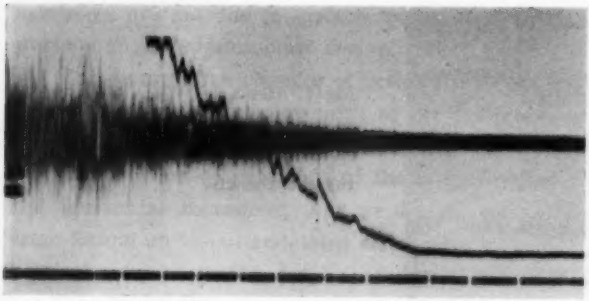

Fig. 2. (Case 1.) EMG of medial rectus. Action potentials decrease as the eye abducts.

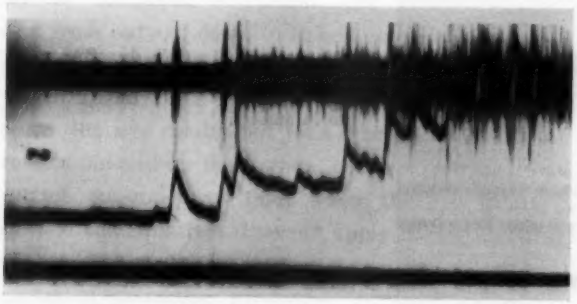

Fig. 3. (Case 2.) EMG of lateral rectus on adduction. Action potentials increase.

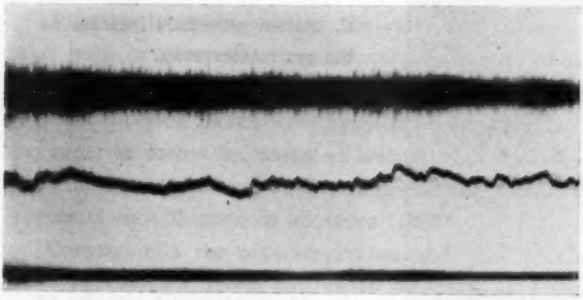

Fig. 4. (Case 2.) EMG of superior rectus on adduction. There is little fluctuation.

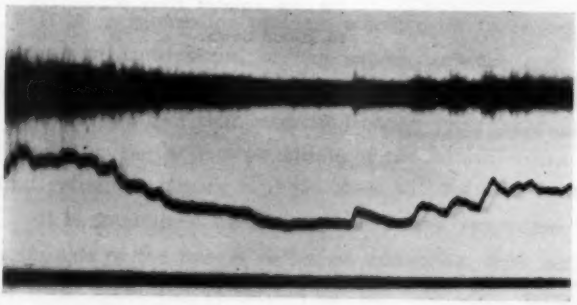

Fig. 5. (Case 2.) EMG of inferior rectus. Action potentials decrease as the eye abducts.

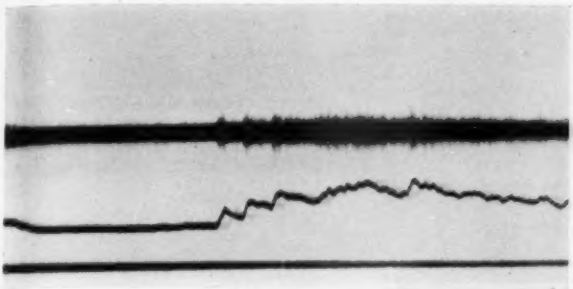

Fig. 6. (Case 2.) EMG of inferior rectus. Action potentials increase as the eye adducts.

the tenotomy of the medial rectus although adduction does not occur. The fact may support the possibility that the superior rectus and the inferior rectus take part in retraction of the eye.

Consequently, the lateral rectus, the superior rectus, and the inferior rectus may take part in retraction. But retraction perhaps does not occur by them alone. It seems that retraction of the eye occurs by both the topographical changes and the abnormal innervation.

Summary

The electromyographic studies of the extraocular muscles of retraction syndrome were performed, and the results were as follows:

1. Action potentials were obtained from the lateral rectus of 12 out of 14 cases, and they were abnormally many on adduction.
2. Abnormal action potentials of the lateral rectus on adduction are not due to stretch reflex, but due to the abnormalities of the fifth nerve nucleus or the supranuclear region.
3. Abnormal spike discharges of single NMU, that is, low amplitude NMU voltage, fibrillation voltage, fasciculation voltage, reinnervation voltage, and grouping voltage were not detected.
4. Action potentials of the medial rectus increased normally on adduction and decreased normally on abduction.
5. Action potentials of the superior rectus and the inferior rectus tend to increase on adduction, but it is not sure since the cases were very few.
6. It seems that retraction occurs by both the topographical changes and the abnormal innervation of the extraocular muscles.

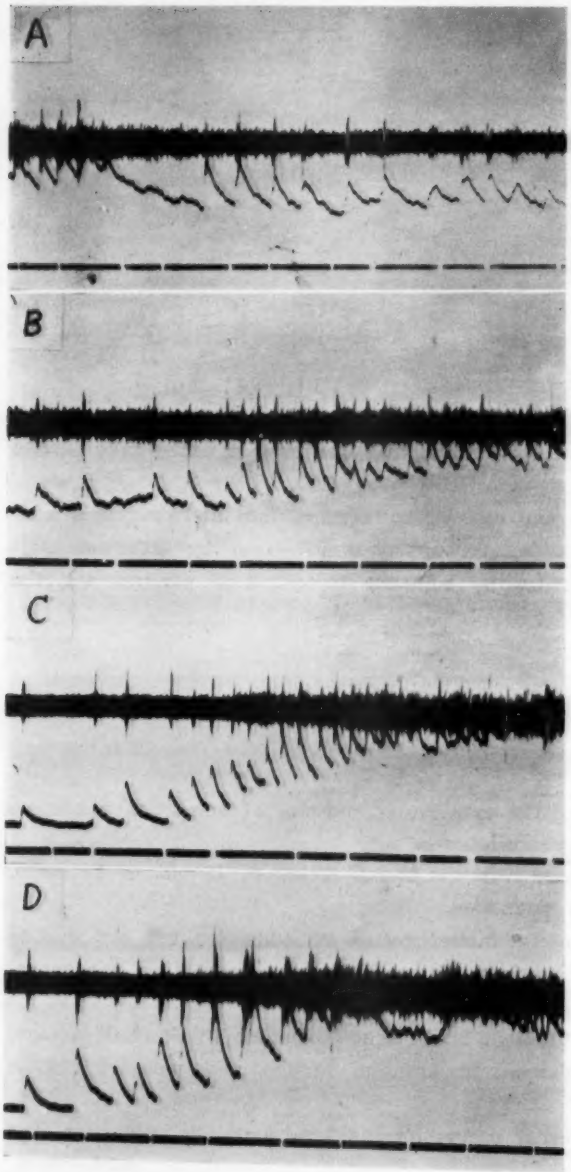

Fig. 7. (Case 3.) EMG of right lateral rectus.
A. Action potentials decrease as the eye adducts.

B. Action potentials increase as the eye rotates from out-and-down to in-and-up.

C. Action potentials increase as the eye rotates up.

D. Action potentials increase as the eye rotates from in-and-down to out-and-up.

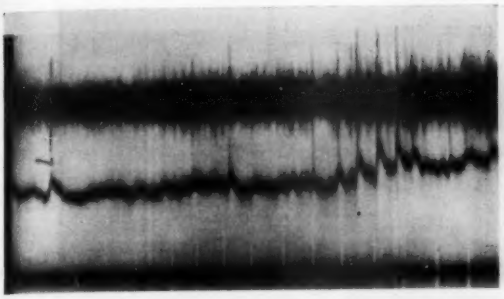

Fig. 8. (Case 3.) EMG of right inferior rectus. Action potentials increase as the eye adducts.

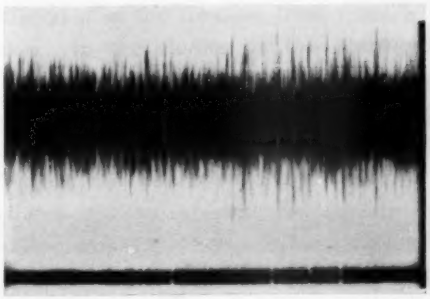

Fig. 9. (Case 5.) EMG of lateral rectus. Action potentials do not increase or decrease as the eye adducts.

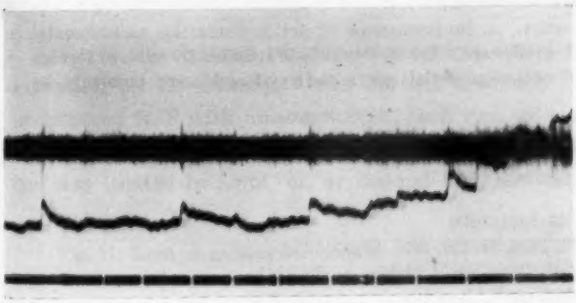

Fig. 10. (Case 5.) EMG of inferior rectus. Action potentials increase as the eye adducts.

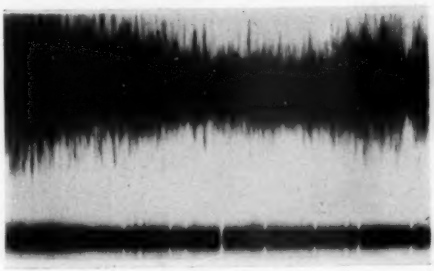

Fig. 11. (Case 6.) EMG of superior rectus. Action potentials decrease as the eye abducts.

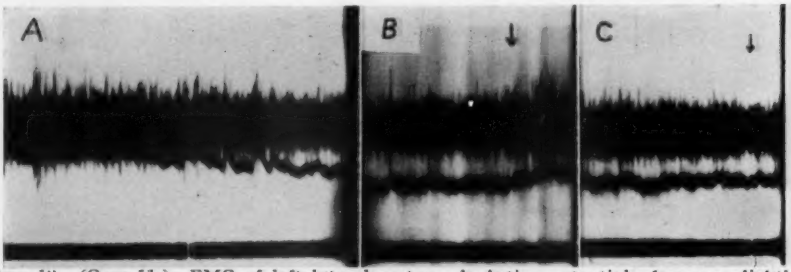

Fig. 12. (Case 11.) EMG of left latercl rectus. A. Action potentials decrease slightly as the eye abducts from adducted position. B. Action potentials increase slightly when the eye which is steadied by fixation forceps is ordered to adduct. C. No fluctuation of action potentials when the eye is adducted passively by fixation forceps.

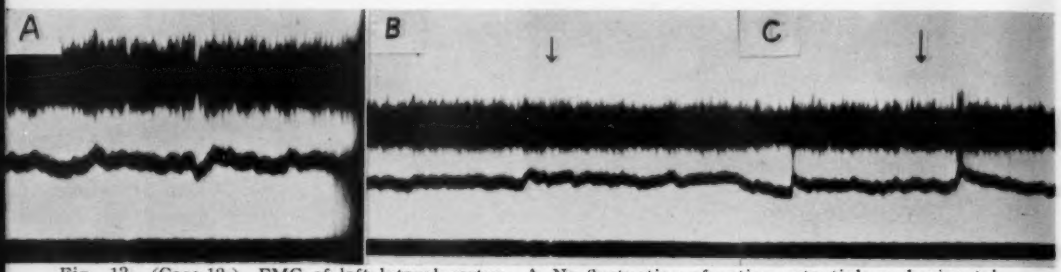

Fig. 13. (Case 12.) EMG of left lateral rectus. A. No fluctuation of action potentials on horizontal movement. B. No fluctuation of action potentials when the eye which is steadied by fixation forceps is ordered to adduct. C. No fluctuation of action potentials when the eye is adducted passively by fixation forceps.

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STUDIES ON THE DEVELOPMENT OF THE DEPTH PERCEPTION FOR NEAR AND DISTANT OBJECT IN THE CHILDHOOD

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As regards the systematic studies on the development of stereopsis in chidren, there is only one treatise by Sachsenweger and Junker[10]. Moreover, nothing is known about the process through which the stereopsis develops. Adachi and Inatomi[1] found recently that the visual acuity in case of amblyopia was generally better in near vision and its improvement started upon the near vision. They considered this fact as endorsing an enlargement of the function from "nearness" in the developmental process of form perception. In the studies on the developmental process of form perception, such a fact is expected to offer a valuable suggestion.

The author attempts to study on the development of the binocular stereopsis with the use of three-rod apparatus for both distant and near vision. Accordingly, although the purpose of the author's study is originally directed to elucidate the process of the development of the binocular stereopsis, the results obtained are considered also to have some relations to the knowledge about the speciality in the spatial perception of children.

Materials

Out of 151 children ranging in age form 4 to 8 years, 134 were subjected, with the exception of 17, namely, 5 who did not show any interests in the test, 3 who couldn't understand it, 3 with anisometropia, each one with convergent or divergent squint and 4 who made always the same answer regardless of the condition of whether the central rod was located in front of or behind the two lateral rods. As controls, 18 adults

Fig. 1. 3-rod apparatus for distant vision.

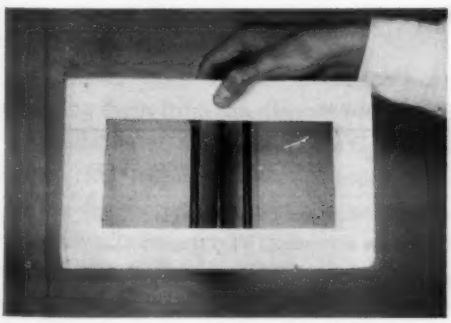
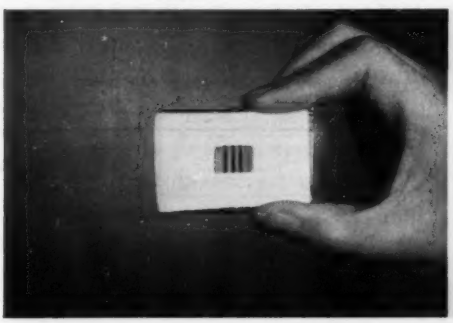


Fig. 2. 3-rod apparatus for near vision.



were selected, 7 with visual acuities of 0.7 up 0.9 and 11 with those of 1.0 or more.

Experimental Apparatus

The size of the main parts of the three-rod apparatus for distant (on the left lines) and for near vision (on the right lines) used in this experiment is shown in Table 1. The figures of the apparatus are shown in Fig. 1 and 2 respectively. In both the apparatus, rods are black colored and are all fixed upright at such stereoscopic threshold as shown in Table 1 and the values converted into the parallax angle are shown in Table 2. Rods can be observed through a window of 1.1×1.6 cm size with a white frame for near vision and of 10.0×20.8 cm for distant vision. These apparatus were operated in front of the white background in the light room. In tensity of the illumination of the background was 100 to 200 lux.

Method

Since the factors connected with the stereoscopic acuity are also considered to exert an influence on the visual acuity, there is a necessity for measuring visual acuity as well as depth perception. Therefore, the measurements of the visual acuity for distant vision and that for near vision were performed, followed by the measurement of the depth perception.

Testing of Visual Acuity. In the measurement of the visual acuity, Landolt's rings of international test type for the distant vision and the Ishiwara's test type for the near vision were always used respectively. The former was used at distance of 5m and the latter at a distance of 30 cm from the eyes. Just before starting the measurement, the author had the children well comprehend what procedures of measuring were being carried out and how to answer.

Measurements of Depth Perception. First of all, the apparatus was hold in the condition in which the central rod was nearer to the subjects than the two lateral rods, this condition being designated by(+)side. And then, when the apparatus was reversed, the central rod turned to be farther than the two lateral rods, this condition being designated by (−) side.

Table 1. Data of 3-rod Apparatus

	observation distance 450 cm	observation distance 50 cm
Thickness of rod	0.7 cm (Visual angle ; 5.4′)	0.08 cm (Visual angle ; 5.5′)
Separation of rods	1.8 cm (Visual angle ; 13.8′)	0.2 cm (Vifual angle ; 13.8′)
Length of rod	10.0 cm (Visual angle ; 1.26°)	1.1 cm (Visual angle ; 1.26°)
Stereoscopic threshold	2.0 cm	0.25 mm

Table 2. Changes in Parallax Angle Accompanied with Changes in Interpupillary Distance at each Age

Age	interpupillay distance (mm)	Parallax Angle φ*	Parallax Angle φ'*
4	53.7	5.4″	5.4″
5	54.8	5.6″	5.6″
6	55.1	5.6″	5.7″
7	56.7	5.8″	5.8″
8	56.7	5.8″	5.8″

*φ: observation distance 450 cm
*φ': observation distance 50 cm

That is, the central rod was always in either advanced or receded position from the lateral rods with a definite stereoscopic threshold (of 2 cm for distant vision and of 0.25 mm for near vision) and with change in the (+) or (−) side of the central rod, the subjects would recognize the central rod as being farther, nearer than the lateral rods, or side by side and according to each recognition they were required to answer with the words "farther", "nearer" or "uncertain".

Children were set to sit in a chair right opposite to the background in front of them and to look ahead of themselves. The apparatus was held and operated at a height of their eyes at distance of 50 or 450 cm ahead. First, they were told to close their eyes, until they open them at the sign of the examiner, to fix their eyes on the central rod and answer with "farther", "nearer" or "uncertain". Immediately after their making an answer, the examiner let them shut their eyes and placed the apparatus in a reverse side or left it as it had been, and then, let them answer again. As preliminary test, such measurements were repeated about 10 times in order to make them learn the correct fixation of their eyes on the central rod.

Such measurements of distant or near vision were repeated 20 times respectively. The order of changing the position of the central rod was (+),(−),(+),(+),(−),(−),(+),(−),(−),(+),(+),(−),(+),(+),(+),(−),(−),(−),(+) and (−). In this experiment, the attention was paid to the following items.

1) Correct fixation of the eyes on the central rod, the manner to judge and to answer are to be previously fully trained.

2) In general, children are apt to expect that the position of the central rod changes alternately like (+),(−),(+),(−).

3) Those who make answer with either (+) or (−) side alone are to be excluded from the experiment.

4) Every effort is to be make for keeping the children interested in the test and for preventing them from the distraction of attention, and that is the most important thing.

Calculation of the Ratio of the Correct Answer. One point is given to every correct answer, zero point to every incorrect answer, and 0.5 point to every "uncertain" answer. So the total sum of the scores in successive 20 answers was obtained, and the ratio of the correct answers was calculated. Supposing that there are 17 correct answers and 2 "uncertain" ones, for example, the ratio of the correct answer is $(17+2\times1/2)/20\times100=90\%$.

Results

The Results of the Visual Acuity Determination. The mean values of the acuity for the distant vision and that for the near vision are presented according to age in Table 3. In Fig. 3 are shown these relationships graphically. It is apparent that the visual acuity becomes better with the growth of children, and the acuity for near vision is better as compared with that for distant vision, this difference being the largest at the age of 4, becoming gradually smaller with advancing age to be scarcely recognizable at the ages of 6 and 7, disappearing at the age of 8.

The Results of the Depth Perception Determination. The age distribution of the ratios of the correct answer in distant vision (F) and in near vision (N) are shown in Fig. 4. That is, with increase in age, the tendency toward a gradual concentration at 100% was observed in both the ratios of the correct answer.

Furthermore, the mean values of the correct answer in near vision and distant vision ($\bar{F}$ and $\bar{N}$) is shown according to age in Table 3. Graphic recording of these data is presented in Fig. 5. As children advance in age, both $\bar{N}$ and $\bar{F}$ become nearer to the adult levels, but in their developmental process, $\bar{N}$ is always better than $\bar{F}$ and the younger the children, the greater is the difference and as they grow up, the difference disappears gradually.

However, besides dealing with the depth perception separately for either distant or near vision, there arises a necessity for the simultaneous considerations of the two functions closely

Table 3.

age	$\bar{V}_f$	$\bar{F}$	$\bar{V}_n$	$\bar{N}$	$\overline{F/N}$
4	0.8_9	70.1	1.1_5	93.2	0.743
5	1.0_0	86.4	1.1_9	97.8	0.812
6	1.2_3	92.4	1.2_7	98.8	0.904
7	1.1_8	95.7	1.1_9	100.0	0.945
8	1.2_8	99.1	1.2_8	109.0	0.991
adult	0.7_8 1.2_1	97.3 99.5	0.7_8 1.2_3	97.3 100	0.995

$\bar{V}_f$, $\bar{V}_n$: The average visual acuity for distant & near vision at each age, respectively.
$\bar{F}$, $\bar{N}$: The average ratio of correct answer for distant & near vision at each age, respectively.
$\overline{F/N}$: The average of F/N at each age.

Fig. 3. The average visual acuity for distant vision($\bar{V}_f$)and for near vision($\bar{V}_n$)at each age.

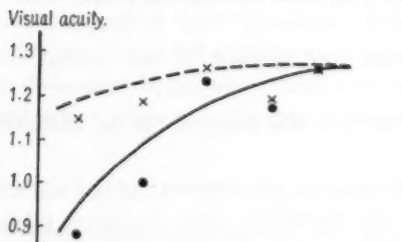


Fig. 4. Distribution of F and N at each age.

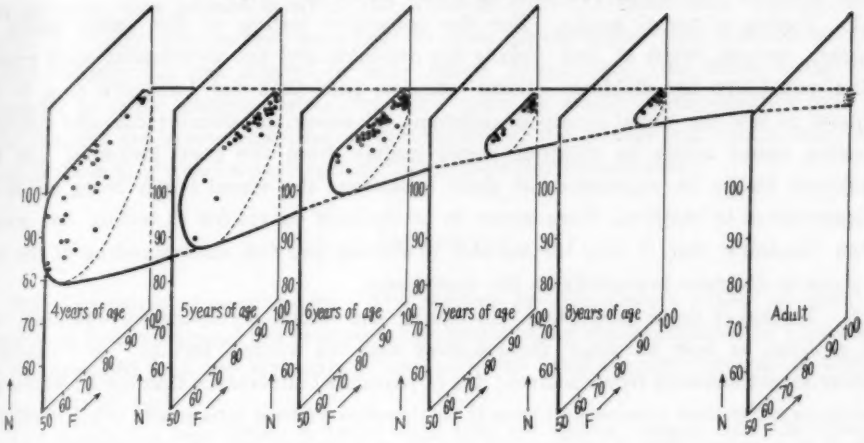


Fig. 5. The average of F and N at each age.

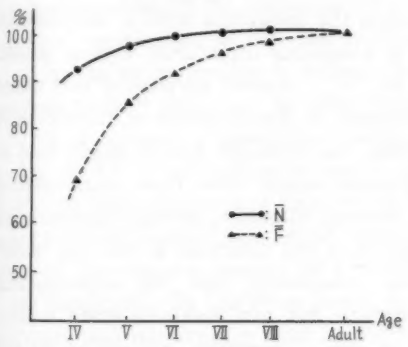
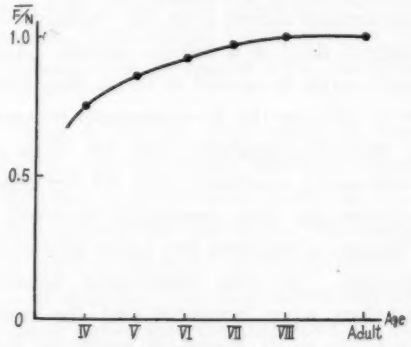


Fig. 6. The average of F/N at each age.



connected with each other. Thereupon, considering that when there was a difference between the ratios of the correct answer in distant vision and that in near vision, the latter was always better in all cases except one, F/N in each case was calculated, the mean values at each age $\overline{(F/N)}$ are shown in Table 3. Graphic recording of this relationscip is presented in Fig. 6. Based on this figure, the stereoscopic acuity is considered to be closely related to age and it seems to be justified to think that the curves show the developmental process of the stereopsis in children ranging in ages from 4 to 8 and the stereoscopic acuity attains the adult level at the age of 8.

Further, the results of the intelligence test performed in these children of 4,5 and 6 years of age revealed that the intelligence quatiant has no correlation with F,N or F/N.

Criticism on the Methods of the Experimentations

1) Testing of visual acuity. For the subjective testing of the visual acuity in children, various types of test objects are available and are investigated with respect to the suitability for children. Some opinions hold that the Landolt's ring is not adequate to test the visual acuity in children. However, considering that the difficulty in testing visual acuity in children comes mainly from the poor judgment and the insufficient ability in expression of their sensation, the visual acuity being apt to be underestimated in children, there seems to be no fault on results of testing by means of the Landolt's ring if only the suitable practicing and full understanding of the test are given to children previously to the experiment.

2) Testing of depth perception. In testing the depth perception of children, the first problem is how to make them answer with the words "farther" or "nearer". In order to get answers from children, it is of particular importance that the experimental procedures excite their interest and keep their attention without fatigue and being tired of it. Since it is apparent that in performing the usual three-rod test, a considerable degree of endurance and mental concentration are required of children, it seems to be almost inevitable that there is a limit to get accurate results in the test. And then taking this fact into consideration, the author planned to use the above-mentioned method. This method differs from the ordinary ones in using the apparatus with a stereoscopic threshold fixed at a definite degree. According to numerous literatures, normal stereoscopic acuity is proved to be ranging from 1.8″ to 7.3″. As the purpose of this study was to observe the developmental process of stereopsis, if the threshold of three-rods in the author's apparatus was too large or too small, the obtained results might lead to an erroneous conclusion that the developmental process might have been accomplished earlier or later than reasonably expected. When interest, attention, mental concentration or fatigue of children are taken into consideration, it is not desirable to use too small threshold. In this experiment however,it was considered that the experimental conditions were favorable and the testing procedure did not make children weary, so a fairly small threshold could be permitted to use. Therefore, the value of the threshold which was set up in the author's apparatus was determined based on the values within the physiological limits reported by many workers. If the pallalax angle in the three-rod apparatus for near vision is set up at 5.4″ which is equivalent to that for the distant vision, the perception is said to be impossible at an observation distance of 50 cm (Matsubayashi[7]), but theoretically, the disparity of the possible perception in case of distant vision may also produce the same effect in case of near vision, in so far as the conditions of the apparatus are the same. If the Matsubayashi's theory is applied to this experiment, N would be expected to be exceedingly bad, actually however, N is better as compared with F, besides varying with age in both N and F. That is, stereoscopic acuity is better in case of near vision.

When the parallax angle set up in the author's apparatus, 5.4″, is used, there

may be some cases present in which the answers with the same side, namely either (+) or (−) only, are correct according to their physiological zero position or their enpiric horopter for the distant or near vision respectively. In these cases, it is impossible to make differentiation from the cases in which psychologically only the same answers are given regardless of the position of the central rod. Such four cases found in the author's series were excluded from the experimental data.

Discussion

The development of visual acuity. The acuities for the distant vision at each age obtained in this experiment are in general agreement to the results reported by Chavasse[5], but as compared with the Watanabe's results[11], its developmental process is delayed by one year of age.

The acuity for near vision is better than that for distant vision and the younger the child, the greater is the difference between the two. Such a superiority of the acuity for near vision over that for distant vision is also recognized in the course of treatment of amblyopia[1] and in case of amblyopia, this fact is said to be accounted for the wakening of the accommodation which have been abandoned. In this experiment, however, explanation of this fact is difficult from the physiological point of view and it seems to be reasonable to believe that the developmental process of form perception expands from "nearness" to "far distance".

The devlopment of stereopsis. As shown in Table 3, in the adult cases of visual acuity of 0.7 to 0.9 (0.78 as average), the averge ratio of the correct answer is equally 97.3% for both distant and near vision and in the adult cases of visual acuity of 1.0 or more (1.21 as average for distant vision and 1.23 as average for near vision) the average ratio of the correct answer is somewhat higher than the former, but the difference is to be neglected. On the other hand, in the cases of children, the visual acuity increases with age and the average ratio of the correct answer shows also the corresponding rise. But, as far as the author's results are concerned, the ratio over 95% is to be expected in adults, with the visual acuity as low as that in children of 4 or 5 years of age, but in children such a high ratio as in adults with the same visual acuity couldn't be obtained. In addition to this, if there were a parallel relationship between the visual acuity and the stereopsis 100% of the correct answer would already be obtained at 5 years of age, but it wasn't the case. Therefore, it seems justified to conclude that the process of the development of the visual acuity is finished a little earlier than that of stereopsis.

Such an opinion may also be supported ontogenetically. Stereopsis is composed of many associated functions but according to deCrinis[6] the site of this function is located mainly in the peristriate area of the visual hemisphere and the axes cylinders in this area are said to become mature later than that in Brodmann's Areas 17 and 18. Accordingly, the development of stereopsis does not run parallel with that of visual acuity

based on the simpler mechanism, but with increase in memorizing capacity, the center of which is always located along the proper sensory center and its development is said to be usually completed at 5, at least at 8 years of age[10].

Judging from the above-mentioned facts that the stereoscopic acuity increases with advancing age, closely connected with the development of memorizing capacity and attains later its full development than the visual acuity. It seems to follow justly that various experiences in the life circle of children will lead the way to the full development of stereopsis. The various complicated sensory functions of the human life will be completed through the various experiences during their childhood, by way of play with tools, and so on, the experiences expanding the life circle of children[4].

When dealing with stereopsis of children, it is necessary to take a psychological factor into account, besides the sensory and physiological factors. According to Sachsenweger and Junker[10], the development of stereopsis has an intimate relationship with attention as well as mental concentration.

With the growth of children, the parallax angle becomes enlarged, accompanied with increase in the interpupillary distance (Table 2), but when the apparatus for near vision is used, the parallax angle is 5.8″ in the case of 8 year-old children whose interpupillary distance of 56.7 mm, while it makes an angle of 5.4″ with an interpupillary distance of 52.7 mm in 4-year old children and such a slight difference as 0.4″ corresponds in stereopscopic acuity to only 0.00087 cm. This can be said to be the same in the apparatus for distant vision. Consequently, so far as the author's experiments are concerned, changes in parallax angle connected with increasing interpupillary distance with age couldn't be regarded as a possible cause of increase in stereoscopic acuity.

Precedence of stereopsis for near vision over for distant vision in its development process. It is said that in the experiment on adults by means of the rod test, stereoscopic acuity generally becomes better in accordance with increase in the observation distance. Since the definite and fixed stereoscopic threshold is set up in the author's method, in those adults showing correct answers of 100% in the rod test for both distant and near vision, the stereoscopic acuity might be better than the ability perceiving the threshold of 5.4″ to 5.8″. Thus, since the author's method cannot determine an exact threshold in each case, it is no wonder that the results of the experiment on adults are not influenced by the observation distance. Stereoscopic perception, however, is said to be essentially produced by disparity. Therefore, theoretically speaking, stereoscopic acuity is expected to change with the disparity regardless of the observation distance, and in adults it is said to be independent of the changes in the observation distance (Ogle[8]).

In this way, despite of various discussions with regard to the effects of the observation distance, no particular attention seems to have been paid to any of effect of convergence accompanied with changes with the observation distance, and the effect of

convergence has been discussed, from the viewpoint of more proprioception caused by fixation movements than observation distance itself. At any rate, as to whether convergence has some influences upon stereoscopic acuity or not, it is still in dispute. Adachi and Inatomi[2] have noticed that the sensation of nearness is closely connected with the hand work, evoking attention which stimulates convergence and they, together with the author, have further presumed, based on encephalographic studies on infants, that convergence stimulating factor may be closely connected with an elevation of the general activity level of the brain induced by the excitement of the Ascending Reticular Activating System[3]. Moreover, Yuge[12] pointed out the convergence as the part of the ocular position controling mechanism, which is maintained by stimuli of various sorts through not only the sensory stimulation, but also psychic tension such as the attention, interest and intention of doing.

According to the results already mentioned, stereoscopic acuity for near vision is almost always better than that for distant vision in children, ranging in age from 4 to 7 years, It seems justified to believe that this fact may originate from the effects of convergence upon the stereoscopic acuity of children, but actually, the above-mentioned considerations lead naturally to the conclusion that it entirely depends on the particulary response of children to "nearness" itself. The fact that stereoscopic acuity varies with advancing age or of the observation distance inspite of the same condition of disparity for both near and distant vision should be explained from the peculiarity of recognition of visual space in children, and this peculiarity may also be related with the superiority of the visual acuity for near vision over that for distant vision.

Children first exhibit their ability of stereoscopic perception for objects near at hand in their living environment and this ability expands to far distant objects, and the preponderance of near vision in the visual space is prerequisite to this process.

In this way, it is reasonable to assume that although the stereopsis in adult is ultimately nothing but a response caused directly by the disparity, regardless of the observation distance[9], it develops from its rudiment through the stage of the preponderance of the near vision over the distant vision to reach the equality between both.

From the above results, the ability to perceive the visual space is considered to be completed at around 8 years of age and according to Chavasse, this is the stage in which visual reflexes as well as fixation reflex are almost unconditionally established.

Conclusion

The development of the depth perception for near and distant object was studied in 134 cases of children ranging from in age 4 to 8 years. At the same time, both near and distant visual acuity were measured respectively. As for the three-rod test, two equipments for distant and near vision were so devised respectively as geometrical conditions were as equal as possible.

As the result of these experiments, the acuity for near vision is completed at the

age of 4, but that for distant vision at the age of 5. This fact is considered to indicate that the development of the form perception in childhood begins first for near objects and then expands to distant objects. Moreover, the development of visual acuity is completed earlier than that of depth perception.

Concerning the children ranging in age from 4 to 7 years, the stereoscopic acuity for near vision is generally better than that for distant. The younger the age, the greater is the difference between the two. Therefore, it is likely that the development of depth perception also begins for near objects and then expands to distant objects. In this way, depth perception seems to be completed at the age of 8. No relationship could be found between the depth perception and intelligence quotient.

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Correction

In the article "Studies on the cellular lesions induced by Adenovirus Type 8 in tissue culture and on those of the conjunctival epithelium of epidemic keratoconjunctivitis" by Dr. S. Sugiura et al. in the July~September issue, 1959, the next sentence "the change of the nuclei corressonds to karyolysis, and in type B" was left out following the term „In type A" in the last line of p. 185.

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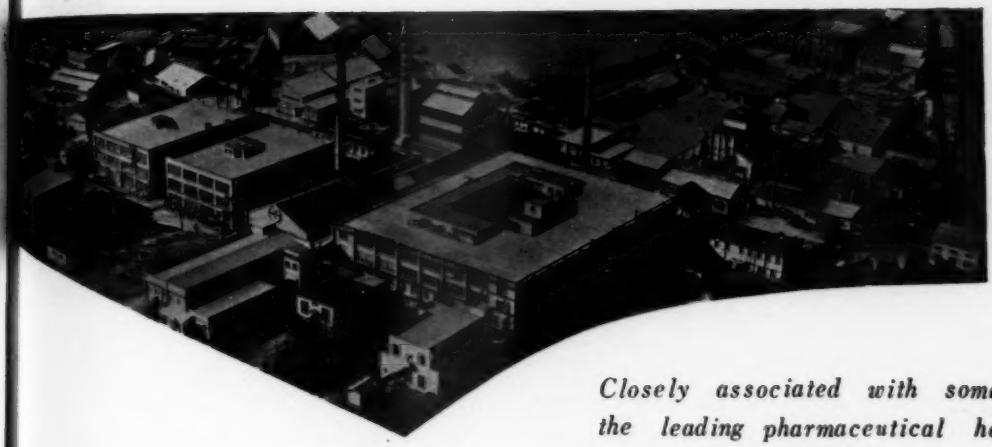

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